SYNTHESIS, CHARACTERIZATION AND ADSORPTION PROPERTIES OF MOLECULARLY IMPRINTED SILICA GEL FOR THE REMOVAL OF 2-HYDROXYBENZOIC ACID (2-HA) FROM AQUEOUS SOLUTION

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KUALA LUMPUR

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DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE

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SYNTHESIS, CHARACTERIZATION AND ADSORPTION PROPERTIES OF MOLECULARLY IMPRINTED SILICA GEL FOR THE REMOVAL OF 2-HA FROM AQUEOUS SOLUTION

ABSTRACT

A molecularly-imprinted silica gel (MISG) sorbent for selective removal of 2-hydroxybenzoic acid (2-HA) was prepared by a surface imprinting technique with a sol gel process. The 2-HA-imprinted silica sorbent was evaluated by parameters including influence of pH, static, kinetic adsorption and selectivity experiments. The optimum pH for maximum adsorption capacity to the 2-HA appeared around pH 2 by the polymer. Also, the imprinted sorbent has a fast uptake kinetics which is obtained within a short shaking period of 5 min. When the initial concentration of 2-HA solution increases from 20 ppm to 400 ppm, the adsorbed amount of 2-HA increases from 8.57 mg/g to 60.95 mg/g. The polymer displays good selectivity, and exhibit good reusability. Equilibrium isotherms have been measured experimentally for the adsorption of 2-HA towards MISG. Freundlich model is fitted well for the description for 2-HA adsorption towards sorbent. The process was spontaneous in nature. Pseudo second-order model provide the best correlation coefficient for adsorption kinetics 2-HA towards MISG sorbent. The thermodynamics parameters such as $\Delta G°$, $\Delta H°$ and $\Delta S°$ values indicated that adsorption of 2-HA towards silica gel was feasible, spontaneous and exothermic in the temperature range of 298K to 353K.

SINTESIS, PENCIRIAN DAN PENJERAPAN GEL SILIKA PENCETAK MOLEKUL UNTUK PENYINGKIRAN 2-HA DARI LARUTAN AKUEUS

ABSTRAK

Penjerap gel silica pencetak molekul untuk penyingkiran selektif 2-HA telah disediakan dengan teknik pencetakan permukaan dengan proses sol gel. Penjerap pencetak silica dinilai oleh parameter termasuk pengaruh pH, statik, penjerapan kinetik dan eksperimen selektiviti. Kebolehan jerapan terhadap 2-HA berlaku di sekitar optimum pH 2 oleh polimer. Selain itu, pencetak penjerap mempunyai kadar penjerapan yang pantas dalam masa 5 minit. Apabila kepekatan larutan 2-HA meningkat dari 20 ppm kepada 400 ppm, kebolehan jerapan 2-HA meningkat dari 8.57 mg g^{-1} kepada 60.95 mg g^{-1}. Polimer menunjukkan selektiviti dan penggunaan semula penjerap yang baik. Keseimbangan isoterma diukur secara eksperimen untuk penjerapan 2-HA terhadap penjerap gel silica pencetak molekul. Model Freundlich sesuai untuk menggambarkan penjerapan 2-HA terhadap penjerap. Proses adalah spontan secara semulajadi. Model pseudo second-order memberikan pekali korelasi yang baik untuk penjerapan kinetik 2-HA terhadap penjerap. Parameter termodinamik seperti nilai $\Delta G°$, $\Delta H°$ and $\Delta S°$ menunjukkan penjerapan 2-HA terhadap gel silica adalah mudah, spontan dan eksoterma dalam suhu antara julat 298K to 353K.

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TABLE OF CONTENTS

		Page
ORIGINAL LITERARY WORKS DECLARATION		iii
ABSTRACT		iv
ABSTRAK		v
ACKNOWLEDGEMENTS		vi
TABLE OF CONTENTS		vii
LIST OF FIGURES		x
LIST OF TABLES		xi
LIST OF ABBREVIATIONS		xii
CHAPTER		
1.0	**INTRODUCTION**	
1.1	Background study	1
1.2	Significance study	3
1.3	Objectives	4
2.0	**LITERATURE REVIEW**	
2.1	Introduction	5
2.2	The concept of imprinting	6
2.3	History of molecular imprinting	7
2.4	Classification of molecular imprinting	9
2.4.1	The covalent approach	9
2.4.2	The non-covalent approach	11
2.4.3	Semi-covalent approach	14
2.5	Factors affecting the imprinting process	16
2.5.1	Template	16
2.5.2	Functional monomer	17
2.5.3	Cross-linkers	19
2.5.4	Porogenic solvents	20
2.5.5	Initiators	20
2.6	Preparation methods of MIP	21
2.6.1	Bulk polymerization	21
2.6.2	Suspension polymerization	21
2.6.3	Precipitation polymerization	22
2.6.4	Multi-step swelling polymerization	22
2.6.5	Surface Imprinting polymerization	23
2.7	Sol gel process	23
2.8	Factors affecting sol gel structure	25
2.8.1	Basic sol gel components	25
2.8.2	Catalyst type and concentration	26
2.9	2-Hydroxybenzoic acid	28
2.10	MIP for the removal of 2-Hydroxybenzoic acid (2-HA)	29

3.0 METHODOLOGY

3.1 Chemical reagents 32
3.2 Instrumentation 33
3.2.1 Fourier Transform Infrared Spectorscopy (FTIR) 33
3.2.2 Scanning Electron Microscopy 33
3.2.3 Surface Area and Porosity 33
3.2.4 Thermal Gravity Analysis (TGA) 33
3.2.5 Ultraviolet-Visible (UV-vis) Spectroscopy 34
3.2.6 High Performance Liquid Chromatography (HPLC) 34
3.3 Calibration curve 35
3.4 Preparation of 2-HA molecularly imprinted silica gel sorbent (2-HA-MISG) 35
3.5 Static and kinetic adsorption tests of 2-HA onto 2-HA-MISG 36
3.5.1 Effect of pH 36
3.5.2 Effect of contact time 36
3.5.3 Effect of initial concentration 36
3.5.4 Determination of adsorption capacity 37
3.6 Selectivity study 37
3.7 Reusability of imprinted sorbent 38
3.8 Application to real samples 39

4.0 RESULTS AND DISCUSSION

4.1 Preparation of 2-HA-MISG 40
4.2 Characterization of 2-HA-MISG 43
4.2.1 IR Spectra 43
4.2.2 TGA Analysis 45
4.2.3 Scanning Electron Microscopy (SEM) 47
4.2.4 Surface area, porosity analysis and BET isotherm of 2-HA-MISG 48
4.3 Evaluation of 2-HA adsorption by 2-HA-MISG 52
4.3.1 Effect of pH 52
4.3.2 Effect of contact time 53
4.3.3 Effect of initial concentration 54
4.3.4 Binding selectivity of the sorbent 55
4.3.5 Reusability 57
4.3.6 Application to real samples 58

4.4 Effect of the temperature and contact time of 2-HA adsorption onto 2-HA-MISG 59
4.4.1 Adsorption kinetics 60
4.4.2 Pseudo first order kinetic model 60
4.4.3 Pseudo second order kinetic model 62
4.5 Effect of the temperature and concentration of 2-HA adsorption onto 2-HA-MISG 64
4.5.1 Adsorption isotherm 65
4.5.2 Langmuir isotherm 66
4.5.3 Freundlich isotherm 67
4.6 Adsorption thermodynamics 70

5.0 CONCLUSIONS 74

6.0 REFERENCES 75

LIST OF FIGURES

Figure		Page
2.1	Schematic presentation of Molecularly Imprinted Polymer (MIP)	7
2.2	Principle of covalent sol gel imprinting	10
2.3	Examples of interaction in non-covalent imprinting	12
2.4	Principle of non-covalent sol gel imprinting	13
2.5	Principle of semi-covalent sol gel imprinting	15
2.6	Selection of monomers used in the non-covalent approach	18
2.7	Chemical structures of selected chemical initiators	19
2.8	Hydrolysis mechanism	27
2.9	Condensation mechanism	28
2.10	2-Hydroxybenzoic aid	28
4.1	Schematic mechanism of 2-HA-MISG preparation	42
4.2	IR spectra of activated silica gel, non-imprinted sorbent and 2-HA imprinted	44
4.3	TGA curves of NISG and 2-HA-MISG	46
4.4	SEM micrographs of NISG and 2-HA-MISG	47
4.5	The IUPAC calssification for adsorption isotherm with x-axis represent the relative pressure and the y-axis denote the amount of gas adsorbed	49
4.6	Nitrogen absorption and desorption of 2-HA-MISG	50
4.7	Effect of pH on 2-HA adsorption onto 2-HA-MISG	53
4.8	Effect of contact time on 2-HA adsorption onto 2-HA-MISG	54
4.9	Effect of initial concentration on 2-HA adsorption onto 2-HA-MISG	55
4.10	Selectivity study on 2-HA, 3-HA, 4-HA and phenol	55
4.11	Effect of contact time on 2-HA adsorption onto 2-HA-MISG at different temperature	59
4.12	The pseudo first-order adsorption kinetics of 2-HA at different temperature	61
4.13	The pseudo second-order adsorption kinetics of 2-HA at different temperature	62
4.14	Effect of concentration on 2-HA adsorption onto 2-HA-MISG at different temperature	65
4.15	Langmuir adsorption isotherm of 2-HA-MISG at different temperature	67
4.16	Freundlich adsorption isotherm of 2-HA-MISG at different temperature	68
4.17	Van’t Hoff plots of 2-HA adsorption onto 2-HA-MISG	71

LIST OF TABLES

Table		Page
3.1	List of reagents used	32
4.1	Porosities of particles determined by BET analysis	51
4.2	Competitive loading of 2-HA, 4-HA, 3-HA and phenol by the imprinted and non-imprinted sorbents	57
4.3	Extraction recyclability through five extraction/stripping cycles	58
4.4	Recovery of 2-HA in real samples at different spiking levels	58
4.5	Kinetic parameters for 2-HA adsorption onto 2-HA-MISG	63
4.6	Isotherm parameters for 2-HA adsorption onto 2-HA-MISG	70
4.7	Van't Hoff plots of 2-HA adsorption onto 2-HA-MISG	73

LIST OF ABBREVIATIONS

AA	Acrylic acid
ABDV	Azobisdimethylvaleronitrile
AIBN	Azobisisobutyronitrile
AMPSA	Acrylamido-(2 methyl)-propane sulfonic acid
AN	Acrylonitrile
APTES	(3-Aminopropyl)triethoxysilane
BET	Brunauer Emmett Teller
BPO	Benzoyl peroxide
DEAEM	*N*,*N*-diethyl aminoethyl methacrylamide
EtOH	Ethanol
FTIR	Fourier Transform Infrared Spectroscopy
GA	Gentisic acid
HAc	Acetic acid
2-HA	2-Hydroxybenzoic acid
2-HEMA	2-Hydroxyethyl methacrylate
3-HA	3-Hydroxybenzoic acid
4-HA	4-Hydroxybenzoic acid
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
MAA	Methacrylic acid
MIP	Molecularly imprinted polymer
MISG	Molecularly imprinted silica gel
MMA	methyl methacrylate
NIP	Non-imprinted polymer
NISG	Non-imprinted silica gel
NVP	*N*-vinylpyrrolidone NVP
SEM	Scanning Electron Microscopy
TEOS	Tetraethoxysilane
TFMAA	2-(trifluoromethyl)-acrylic acid
UV-Vis	Ultraviolet-Visible
2-VP	2-vinylpyridine
4-VP	4-vinylpyridine

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The quality of the environment is degrading continuously due to accumulation of various undesirable constituents such as pollutant. Pollutants are often materials which are naturally present in the environment with their adverse effects being caused by concentrations higher than those which would be expected from natural causes. As part of a proactive approach to environmental protection, emerging issues with potential impact on human health and the environment is the subject of ongoing investigation. One emerging issue concerns pharmaceuticals and personal care products in the environment and their possible impact on ecosystems (Roger, 2002).

The presence variety of man-made trace pollutants such as in cosmetic products, pesticide, dyes and many more led to harmful on environments and human beings. Nowadays, the pollutant like 2-hydroxybenzoic acid (2-HA) is considered emerging contaminants because relatively little is known about their occurrence, fate, and transport in the environment that could have great side effects on the human and environment (Fuh *et al.,* 2005). 2-HA can be employed as preservative or active ingredient in cosmetic products at low concentration while it will bring serious environmental problems at high concentration (Huang *et al.,* 2011). Therefore, the

efficient removal and determination of 2-HA from aqueous solution has received considerable attentions.

Molecularly imprinted polymers (MIPs) are synthetic materials which can selectively recognize a target molecule or related analogous compounds (Turiel and Martin-Esteban, 2004). In common preparation, a monomer forms a complex with a template and then joined by using cross-linker agent. Removal of the template by extraction will leave the binding site which is complementary to the target analyte in size, shape and functionality (He *et al.,* 2007). MIPs have been widely used as separation media in liquid chromatography (Huang *et al.,* 2003a), sensors (Haupt and Mosbach, 2000), catalysis (Wulff, 2001) and screening (Ye and Mosbach, 2001).

Up to date, amorphous metal oxide sol gel materials have not demonstrated the same degree of success as acrylic based imprinted polymers for chemical applications. However, there are several advantages associated with the use of sol gel materials over polymers. For example, the low thermal stability of polymeric system restricts the method of the template removal to liquid-liquid extraction. This allows a small amount of residual imprint to remain in the polymer matrix which may mislead the mechanism of molecular recognition in the resulting imprinted material. In the case of sol gel materials, the template may be removed by more forceful techniques such as combustion. Also, control of the thickness, porosity and surface area are easier while selectivity and diffusion are comparable and even better than acrylic polymer based imprinted materials (Marx and Liron, 2001). Other than that, the sol gel materials exhibit several advantages, such as large surface areas, more accessible sites, fast binding kinetics, high adsorption capacity and high selectivity (Na *et al.,* 2006).

Studies on MIPs towards 2-HA as a template are well documented over the past years by bulk polymerization and emulsion polymerization (Zhang *et al.,* 2001; Zhang *et al.,* 2002; Li *et al.,* 2008). Most of these materials exhibit high affinity and selectivity (Turiel and Martin-Esteban, 2010) however possessed low binding capacity and poor site accessibility to target analyte (Baggiani *et al.,* 2007). Therefore, the molecular imprinted silica gel (MISG) material has been introduced in order to overcome the drawback of traditional methods (Zhu *et al.,* 2010). Recently, MISG materials have been extensively studied due to the ease of preparation (Lee *et al.,* 2010; He *et al.,* 2008; Yin *et al.,* 2012).

Based on our knowledge, there is no report on studies of MISG towards 2-HA. Herein, we reported highly selective imprinted silica gel sorbent with binding sites situated at the surface for selective removal and separation of 2-HA.

1.2 Significance of Study

The research consisting of synthesizes of 2-HA molecularly imprinted silica gel sorbent (2-HA-MISG) which was targeted for the removal of 2-HA. The research also focused on the characterization of 2-HA-MISG by Fourier Transform Infrared Spectroscopy (FTIR), Brunauer-Emmett-Teller (BET) and Scanning Electron Microscopy (SEM) analyses. The adsorption was evaluated by studying the effect of pH, shaking time and concentrations of 2-HA. Selectivity and reusability was also taken into account as it can affect the removal of 2-HA in aqueous solution. The 2-HA-MISG sorbent material was applied to real samples.

Equilibrium isotherms was measured experimentally and the data analyzed by using the Langmuir and Freundlich model with linearized correlation coefficient at different temperatures. Also, the kinetic model (pseudo first-order and pseudo second-order) was evaluated for the adsorption of 2-HA by 2-HA-MISG. The thermodynamics

parameter such as ΔG°, ΔH° and ΔS° were used to evaluate the effect of temperature on the adsorption of 2-HA by 2-HA-MISG.

1.3 Objectives

The objectives of the study are:

1) To synthesize molecularly imprinted polymer silica gel (MISG) for the removal of 2-Hydroxybenzoic acid (2-HA).
2) To characterize the 2-HA-MISG using Fourier-transform Infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM), thermal gravimetric analysis (TGA), surface area and porosity analysis (BET)
3) To evaluate the 2-HA-MISG for adsorption of 2-HA through effect of pH and the study of kinetic, isotherm, selectivity and reusability.
4) To apply 2-HA-MISG sorbent for removal of real samples

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Molecular recognition is one of the basic process in nature. It can be envisaged as the preferential binding of a molecule to a receptor with high selectivity over its close structural analogues. The dreams of many synthetic chemists have been and continue to be the attempt to translate the principles of biological molecular recognition to abiotic derived materials (Diaz-Garcia and Lainno, 2005). The earliest synthetic approaches to preparing nanostructure materials for molecular recognition and explaining the operation of the human immune system were inspired by the contributions of Mudd in the 1930s (Mudd, 1932) and Pauling in the 1940s (Pauling, 1940). Their basic suppositions were that in living systems, antibodies are constructed by using molecules as imprints or templates. The primary structure of any antibody would be the same, but selectivity should arise from differences in the conformation of the antibody induced by the template molecule. When the target is removed, a cavity with morphological and stereochemical features related to those of the template is maintained to give the antibody a propensity to rebind the molecule. We know this process as molecular imprinting. The description was very similar to the ‘‘lock and key’’ analogy used to

explain the action of enzymes, the biological molecules responsible for hastening and directing biochemical reactions. Although Pauling's theory of antibody formation was later disproved, several groups subsequently tried to apply it to synthetic systems (Diaz-Garcia and Lainno, 2005).

2.2 The concept of imprinting

Molecular imprinting is a technique whereby selective recognition sites can be created in synthetic polymers. There are three approaches to molecular imprinting which are covalent, non-covalent and semi covalent imprinting. The crucial part of all these procedures is to ensure that functional groups of the template molecule fully interact with complementary functional groups of the polymer. Molecular imprinting is a process by which functional and cross-linking monomers are copolymerized in the presence of the target analyte, which acts as a template. Subsequent removal of the template leaves behind binding sites that are complemented to the target analyte in the resultant MIPs.

A schematic representation of the MIP process is shown in Figure 2.1 in which the formation of reversible interactions between the template and polymerizable functionality may involve one or more of the following interactions: [(A) reversible covalent bond(s), (B) covalently attached polymerizable binding groups that are activated for non-covalent interaction by template cleavage, (C) electrostatic interactions, (D) hydrophobic or van der Waals interactions or (E) co-ordination with a metal centre; each formed with complementary functional groups or structural elements of the template, (a-e) respectively]. A subsequent polymerization in the presence of crosslinker(s), a cross-linking reaction or other process, results in the formation of an insoluble matrix (which itself can contribute to recognition through steric, van der Waals and even electrostatic interactions) in which the template sites reside. Template is then removed from the polymer through disruption of polymer-template interactions,

and extraction from the matrix. The template, or analogues thereof, may then be selectively rebound by the polymer in the sites vacated by template, the 'imprints'.

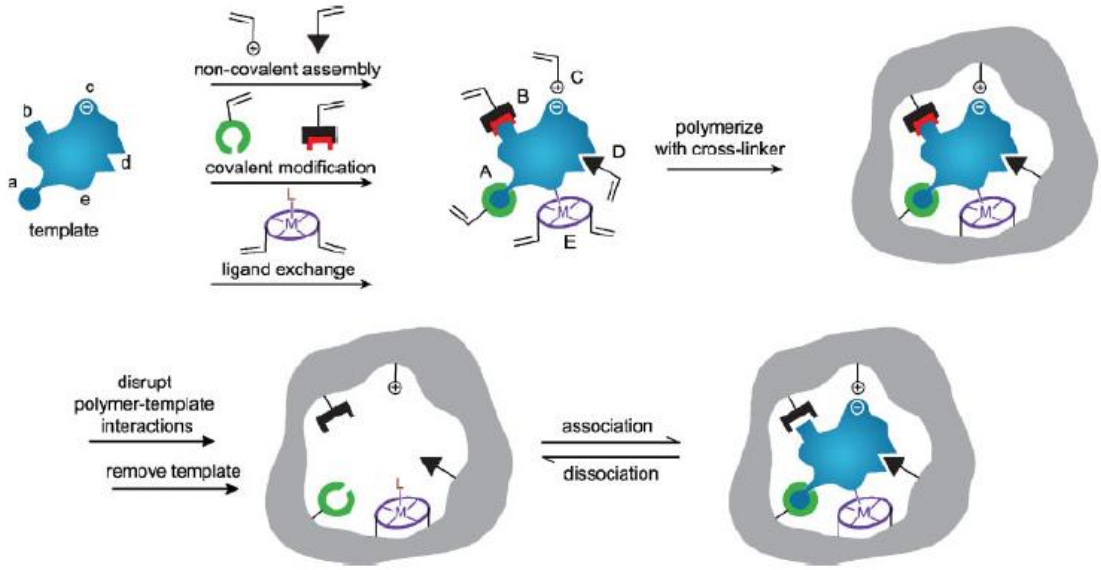


Figure 2.1: Schematic presentation of Molecularly Imprinted Polymer (MIP) (Alexander *et al.*, 2006)

2.3 History of molecular imprinting

In reviewing the historical origins of molecular imprinting as a technique, it is noted that the imprinting was first introduced in the early 1930s by a Soviet chemist M.V. Polykov who performed a series of investigations on the silica for use in chromatography (Polyakov, 1931). It was observed that when silica gels were prepared in the presence of a solvent additive the resulting silica demonstrated preferential binding capacity for that solvent. It was the first time that experiments of this kind were accompanied by explanations of this nature. The mechanism proposed by Polykov was largely overlooked by the scientific community.

In 1949, a study was performed by Frank Dickey, which involved the development of molecular imprinting in silica matrices in the presence of dyes (Dickey, 1949). Dickey observed that after removal of the 'patterning' dye the silica would rebind the same dye in preference to the others. Dickey's silicas can be considered to be the first imprinted materials. Dickey approach is to introduce the template in the sodium silicate pre-polymerisation mixture produced a more definite influence on the structure of the silica whereas Polyakov introduced the template after the silica framework had been formed. Dickey's work is similar to present methodologies, thus, this method became the most widely used in subsequent studies.

Imprinting of silica continued during the 1950s and 1960s. However, the number of publications in the area remains low. Work in the area involved attempting to use imprinted materials for practical separations such as solid phases in chromatography and in thin layer chromatography. The reasons for the limited interest were related to limitations in the stability and reproducibility of the imprinted silica materials. However, the re-emergence of silica based MIP research has occurred. For example, the use of imprinted silicas as solid phases in chromatography was described by Pinel and the co-workers who reported the use of imprinted metal oxide sol-gels to resolve enantiomers (Pinel *et al.*, 1997).

2.4 Classification of molecular imprinting

2.4.1 The covalent approach

Covalent imprinting is one of the polymerisable derivatives in which the template monomer complex is co-polymerised with a cross-linking monomer that was pioneered by Wulff and co-workers (Wulff *et al.,* 1973; Wulff *et al.,* 1977). These derivatives are obtained by forming covalent bonds between template and suitable monomers to produce an 'exact fit' recognition sites, in which the same chemical bonds in the initial template monomer complex reform during any subsequent binding of the imprinted polymer cast. In order to remove the template from the polymer and free up the binding sites, these covalent bonds have to be chemically cleaved. The functionality remaining in the binding site is capable of binding the target molecule by re-establishment of the covalent bond. Only a restricted range of functional groups (alcohol, diols), aldehydes, ketones, primary amines and carboxylic acids can be imprinted by this approach. The advantages of the covalent imprinting method is that the reversible bond between the functional groups is only associated with the template site, and the functional groups responsible for binding are only located in the binding cavities, therefore restricting non-specific binding effects.

Application of sol gel in covalent approach usually involving a prior chemical synthesis step to link the precursors to the template or to a structurally similar molecule to create a 'sacrificial spacer. The example is illustrated in Figure 2.2. This conjugate is then polymerized using an excess of the metal oxide precursor. Once the sol gel is formed, the sacrificial spacer or the template is chemically removed, leaving a pocket that should have the ability to bind molecules of the appropriate size and shape.

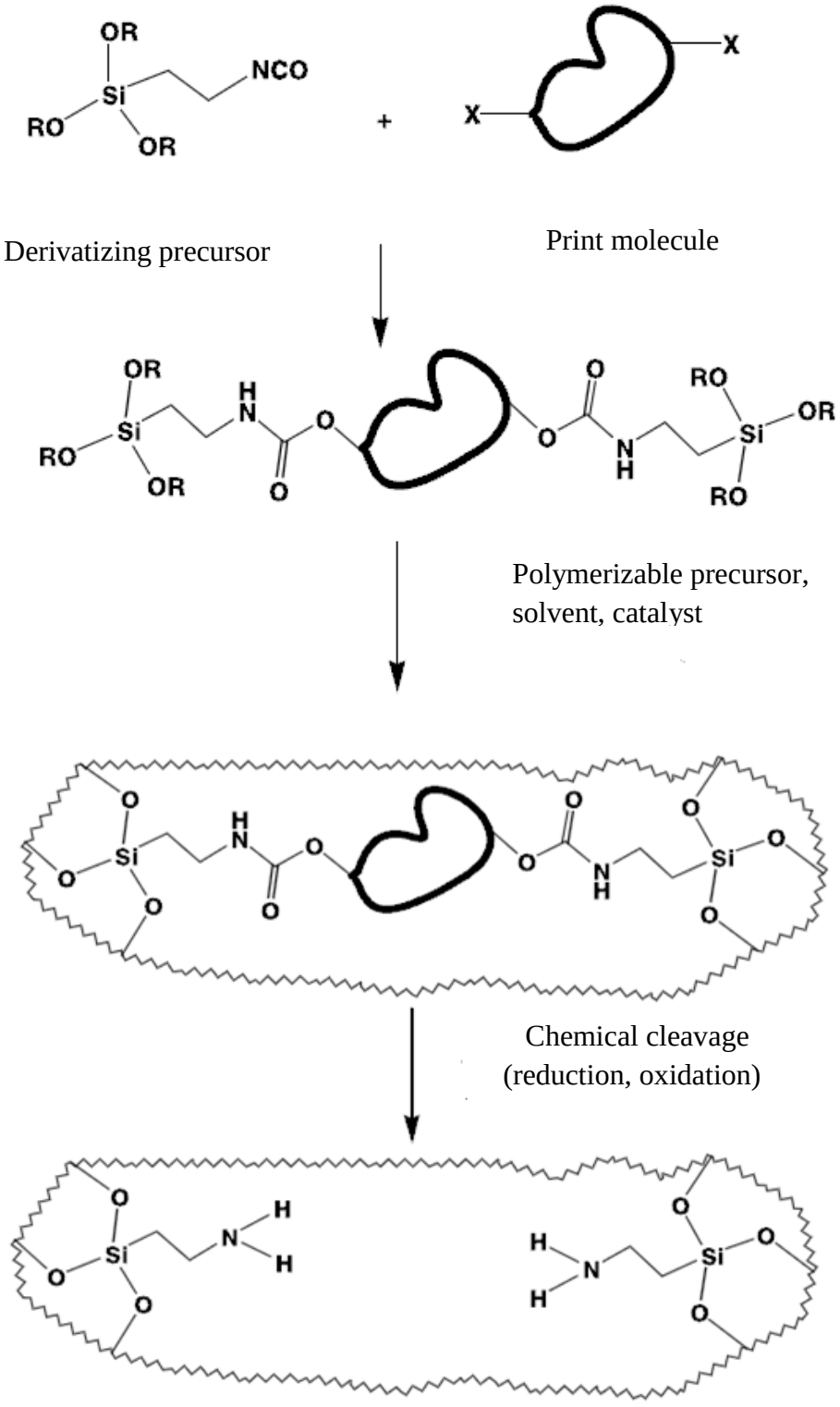


Figure 2.2: Principle of covalent sol gel imprinting (Diaz-Garcia and Lainno, 2005)

2.4.2 The non-covalent approach

Development of non-covalent imprinting by Arshady and Mosbach (1981) using non-covalent forces such as ionic interactions, hydrogen bonding, and dipole-dipole interactions. This approach depends on the formation of pre-polymerisation complex between monomers to suitable functional groups and the template. A host guest relationship is produced due to complementary of the binding sites between polymer and the template. The non-covalent approach is most frequently used for imprinting a wide range of templates. Due to the simple processes for template extraction, a greater number of higher affinity sites are generated and the need to chemically cleave and reform covalent bonds in the covalent process is not required in the non-covalent approach.

The advantage of non-covalent imprinting is due to the simplicity and versatility of the method. This can address elements of the template structure that are not influenced by covalent imprinting by using a range of chemical interactions (ionic, dipolar and hydrogen bonding) as shown in Figure 2.3 . The limitation of this approach is obtaining the variety of binding sites as a result of the assembly of complexation form between monomers and templates during the initial stages of polymerizations. Another limit is set by the peculiar molecular recognition conditions. Most of the formation of interactions between monomers and the template are stabilized under hydrophobic environments while polar environments disrupt them easily (Yan and Row, 2006).

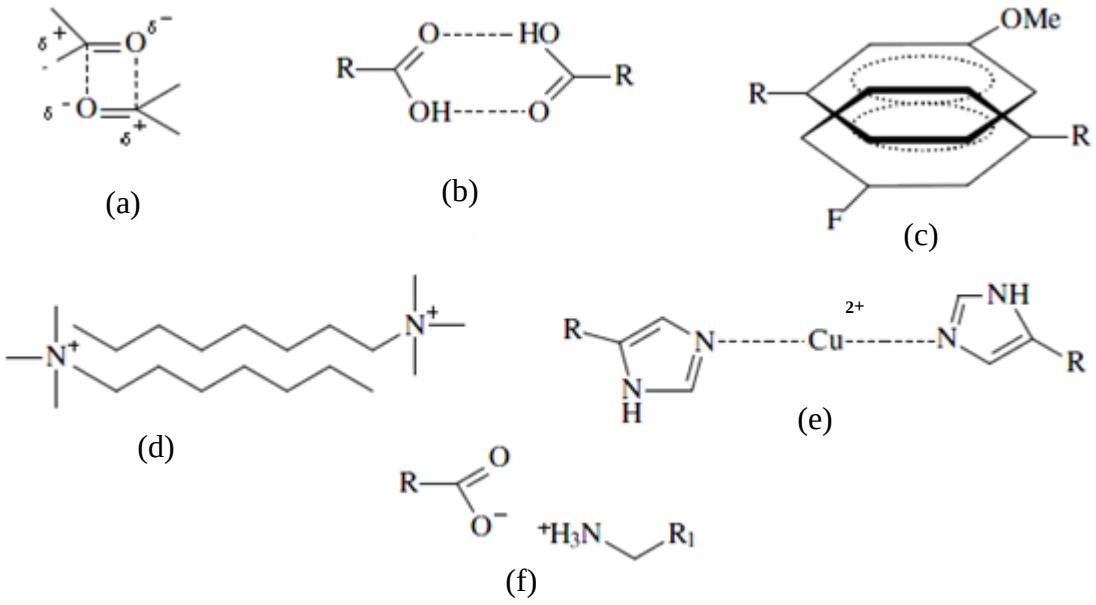


Figure 2.3: Examples of interaction in non-covalent imprinting. (a) electrostatic:dipole-dipole (b) hydrogen bonding (c) π-π stacking (d) van der waals (e) coordination bond (f) electrostatic ion (Spivak, 2005)

In non-covalent approach of molecularly imprinted sol gel, the template may be directly added to a sol gel solution prior to acid-catalyst hydrolysis and condensation. Imprinted sites are generated by van der Waals, π-π stacking stacking, electrostatic and etc. (interactions between the template and the sol-gel network) by using a non-polar sol gel precursor and fairly polar solvent such as ethanol.

As the solvent evaporated to yield a solid porous material, the imprinted sites could be formed by the template's affinity for the sol-gel matrix. The precursor should be carefully selected to provide the porosity necessary to facilitate diffusion of the template into and out of the sol gel. Following the drying step, the gels should be extracted with an adequate solvent to remove the template as shown in Figure 2.4.

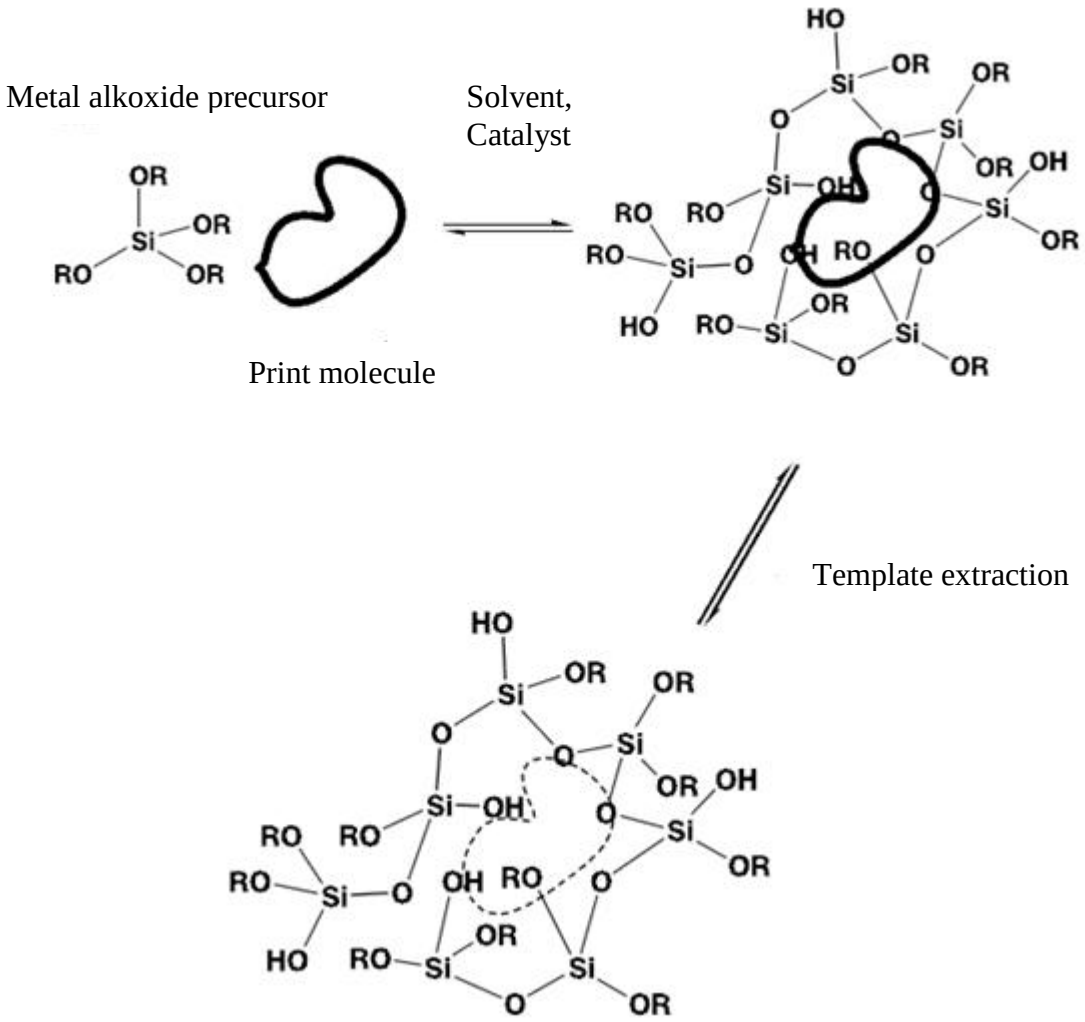


Figure 2.4: Principle of non covalent sol gel imprinting (Diaz-Garcia and Lainno, 2005)

2.4.3 Semi-covalent approach

The purpose of semi-covalent approach is to unite the advantages of the covalent and non-covalent approach. The template is covalently bound to a polymerisable group, the functionality is recovered after cleavage of the templates found in the binding site, and rebinding takes place through non-covalent interactions. A version of this approach is based on the copolymerization of vinyl metal oxide precursor derivatives with methacrylic acid (or related amides) prior to the sol gel process. Hydrolysis of the alkoxy groups in the presence of the template, followed by co-condensation with a metal alkoxide, results in the formation of highly cross-linked hybrid materials.

During the hydrolysis-condensation-crosslinking processes, the imprint molecule organizes itself onto the cavities of the amorphous material. The removal of the template leaves specific receptor sites capable of rebinding the template molecules (Diaz-Garcia and Lainno, 2005). Figure 2.5 shows that the principle of semi-covalent sol gel imprinting.

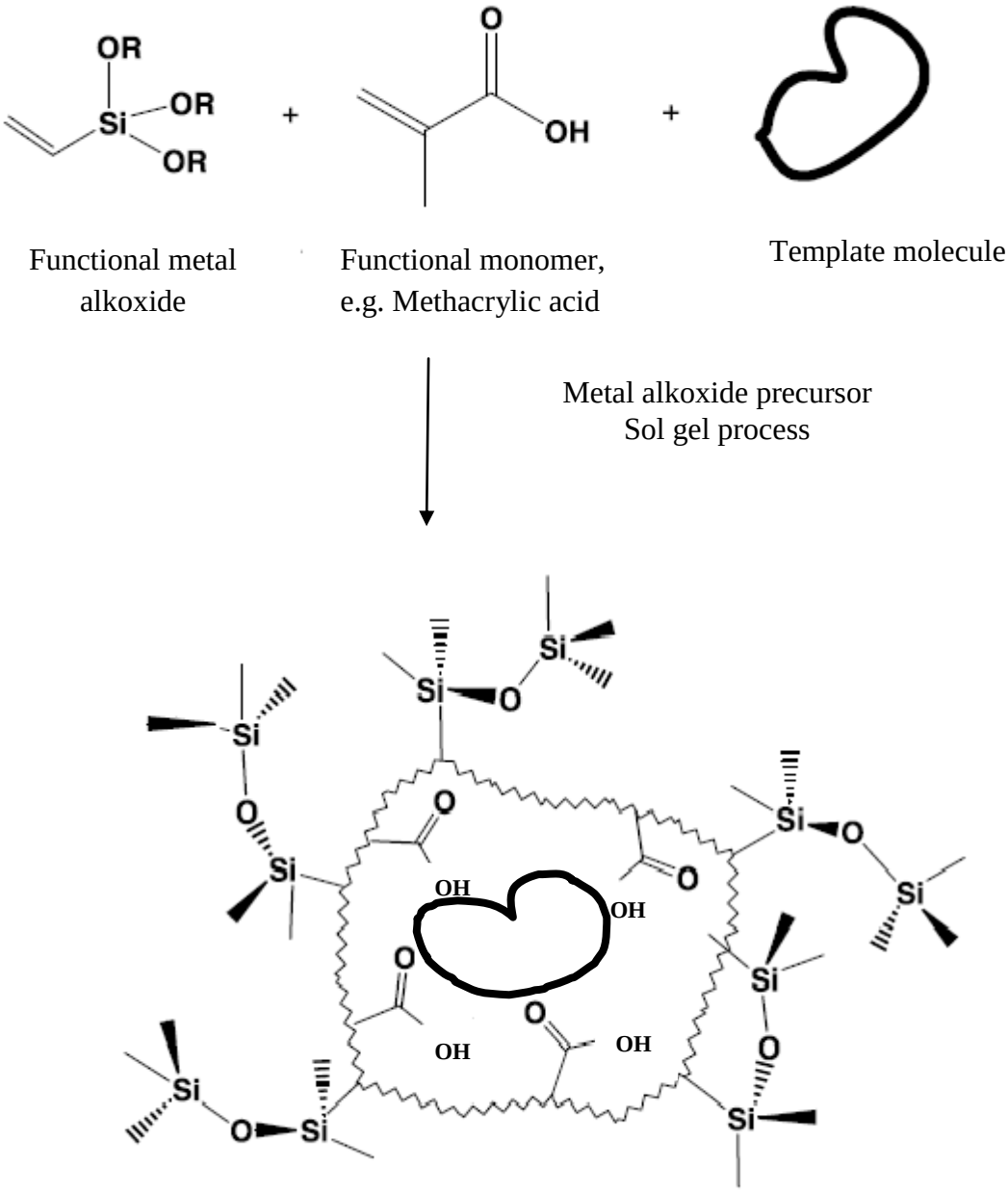


Figure 2.5: Principle of semi-covalent sol gel imprinting (Diaz-Garcia and Lainno, 2005).

2.5 Factors affecting the imprinting process

The synthesis of molecularly imprinted polymers is chemically complex and needs a good understanding of chemical equilibrium, molecular recognition theory, thermodynamics and polymer chemistry in order to ensure a high level of molecular recognition (Yilmaz *et al.*, 2007; Collinson, 1999; Hench and West, 1990a; Avnir *et al.*, 1994; Corriu and Leclercq, 1996). The rational design of MIPs is very complicated due to a number of experimental variables, for example the template, functional monomer, ratio of functional monomer to crosslinker, pre-polymerisation interaction, solvent, initiator, thermodynamic considerations, temperature, pressure, methodology and polymerization parameters. Binding site orientation, stability and accessibility are influenced by the structural characteristics of the polymer matrix. Due to this reason, investigating and optimizing various parameters are essential in order to maximize recognition effects.

2.5.1 Template

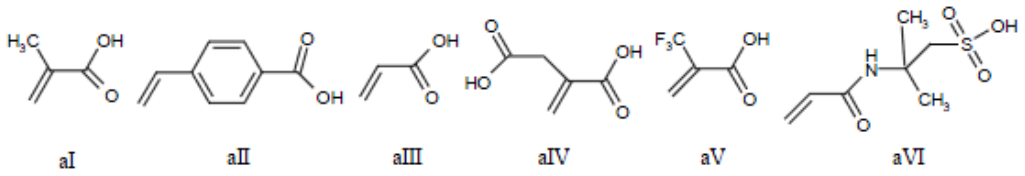
The template is of central importance which directs the organization of the functional groups pendent to the functional monomers. Unfortunately, due to a variety reasons, not all templates are directly amenable to template. In order to be compatible with free radical polymerization, templates should ideally be chemically inert under the polymerization conditions, thus alternative imprinting strategies may have to be sought if the template can participate in radical reactions or is for any other reason unstable under the polymerization conditions. The following are legitimate questions to ask of a template : (1) Does the template possess any polymerisable groups? (2) Does the template posses functionality that could be potentially inhibit or retard a free radical polymerization, (e.g. at around 60 ˚C if Azobisisobutyronitrile (AIBN) is being used as

the chemical initiator) or upon exposure to UV irradiation? (Cormack and Elorza, 2004).

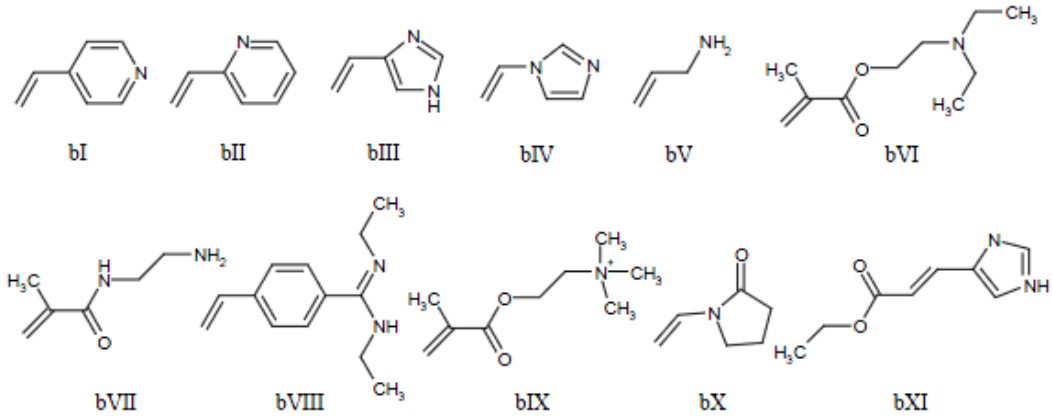
2.5.2 Functional monomer

The careful choice of functional monomer is important to provide complementary interactions with the template and substrates. For covalent molecular imprinting, the effects of changing the template to functional monomer ratio is not necessary because the template directs the number of functional monomers that can be covalently attached; furthermore, the functional monomers are attached in a stoichiometric manner. For non-covalent imprinting, the optimal template /monomer ratio is achieved empirically by evaluating several polymers made with different formulations with increasing template (Kim and Spivak, 2003). It is clearly very important to match the functionality of the template with the functionality of the functional monomer in a complementary fashion (e.g. H-bond donor with H-bond acceptor) in order to maximise complex formation and thus the imprinting effect (Cormack and Elorza, 2004). The chemical structures of a selection of functional monomers are shown in Figure 2.6.

Acidic (a)



Basic (b)



Neutral (c)

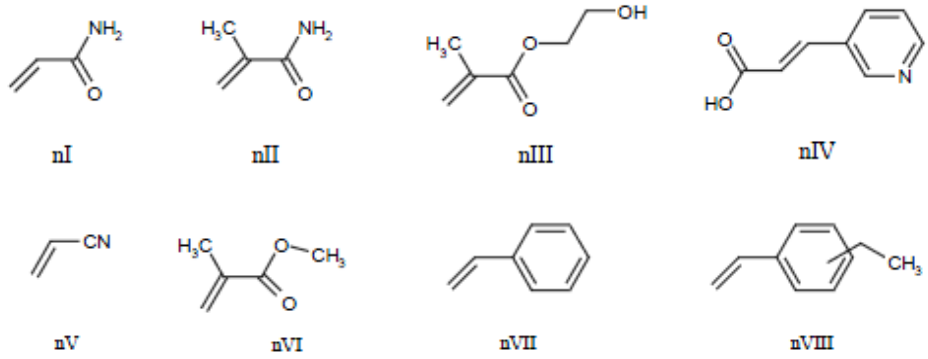


Figure 2.6: Selection of monomers used in the non-covalent approach. Acidic; aI: methacrylic acid (MAA); aII: *p*-vinylbenzoic acid; aIII: acrylic acid (AA); aIV: itaconic acid; aV: 2-(trifluoromethyl)-acrylic acid (TFMAA); aVI: acrylamido-2-methylpropane sulfonic acid (AMPSA). Basic; bI: 4-vinylpyridine (4-VP); bII: 2-vinylpyridine (2-VP); bIII: 4-(5)-vinylimidazole; bIV: 1-vinylimidazole; bV:allylamine; bVI: *N*,*N*-diethyl aminoethyl methacrylamide (DEAEM), bVII: *N*-(2-aminethyl)-methacrylamide; bVIII: *N*,*N*-diethyl-4-styrylamidine; bIX: *N*,*N*,*N*, trimethyl aminoethylmethacrylate bX: *N*-vinylpyrrolidone (NVP); bXI: urocanic ethyl ester. Neutral; nI: acrylamide; nII: methacrylamide; nIII: 2-hydroxyethyl methacrylate (2-HEMA); nIV: trans-3-(3-pyridyl)-acrylic acid; nV: acrylonitrile (AN); nVI: methyl methacrylate (MMA); nVII: styrene; nVIII: ethylstyrene (Cormack and Elorza, 2004).

2.5.3 Cross-linkers

The importance of the cross-linker in imprinted polymer is discussed as follows. First, the cross-linker is important to control the morphology of the polymer matrix whether it can be gel-type, macroporous or a microgel powder. Secondly, it serves to stabilise the imprinted binding site. Also, it imparts mechanical stability to the polymer matrix. From a polymerization point of view, high cross-link ratios are generally preferred in order to access permanently porous (macroporous) materials and in order to be able to generate materials with adequate mechanical stability. So the amount of cross-linker should be high enough to maintain the stability of the recognition sites. Polymers with cross-link ratios in excess of 80% are often used (Cormack and Elorza, 2004). The chemical structures of several well-known cross-linkers are shown in Figure 2.7.

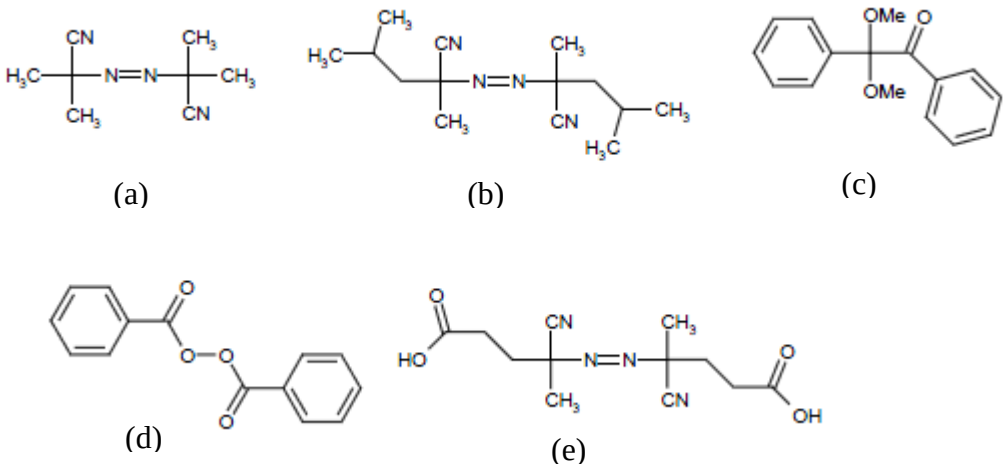


Figure 2.7: Chemical structures of selected chemical initiators: (a) azobisisobutyronitrile (AIBN); (b) azobisdimethylvaleronitrile (ABDV); (c) dimethylacetal of benzil; (d) benzoyl peroxide (BPO); (e) 4,4-azo(4-cyanovaleric acid) (Cormack and Elorza, 2004).

2.5.4 Porogenic solvents

The solvent serves to bring all the components in the polymerization process, i.e. template, functional monomer(s), cross-linker and initiator into one phase. Additionaly, it serves a second important function which is responsible for creating the pores in macroporous polymers. For this reason, it is quite common to refer to the solvent as the "porogen". When macroporous polymers are being prepared, the nature and the level of the porogen can be used to control the morphology and the total pore volume. More specifically, the use of a thermodynamically good solvent tends to lead to polymers with well developed pore structures and high specific surface areas, use of a thermodynamically poor solvent leads to polymers with poorly developed pore structures and low specific surface areas. Increasing the volume of solvent increases the pore volume (Cormack and Elorza, 2004).

2.5.5 Initiators

In principle, any of the methods of initiation described earlier can be used to initiate free radical polymerisation in the presence of templates. However, there may will be priorities for selecting one over another arising from the system under study. For example, if the template is photochemically or thermally unstable then initiators that can be triggered photochemically and thermally respectively would not be attractive. Meanwhile complexation is driven by hydrogen bonding. Then, lower polymerisation temperatures are preferred, and under such circumstances photochemically active initiators may well be preferred as these can operate efficiently at low temperature (Spivak, 2005 ; Cormack and Elorza, 2004).

2.6 Preparation methods of MIP

2.6.1 Bulk Polymerization

MIPs can be prepared in a variety of physical form to suit the final application desired. All the components involved in this polymerization technique are dissolved in a low volume of a suitable solvent (porogen) and left to polymerize thus creating a monolithic polymer (Beltran *et al.,* 2010). The advantage of this method is fast, simple in its execution and no specialized equipment (Yan and Row, 2006). However, the method presents many drawbacks. First, the particles obtained after the last sieving step are an irregular in size and shape, the process is laborious and time consuming. Also, due to its exothermical nature, this method cannot be scaled-up without danger of sample overheating (Yan and Row, 2006).

2.6.2 Suspension polymerization

This method can be used to obtain spherical particles. In this method, all the components involved in the polymerization process are dissolved together in an appropriate organic solvent and this solution is further added to a larger volume of an immiscible solvent. This system is then vigorously stirred in order to form droplets and then the polymerization reaction is induced (Beltran et al., 2010). To improve the spherical beads, suspension polymerization preparation has been reported, where water is used as a continuous phase to suspend a droplet of pre-polymerization mixtures in the presence of a stabilizer (e.g., polyvinyl alcohol) or surfactant. Basically, an organic-based medium (monomers in organic solvent) is mixed with an excess of water containing a suitable suspension stabilizer. Then, the two phases are vigorously mixed by stirring to form a suspension of organic droplets in the aqueous phase with the final bead size being dependent on the size of the droplet (Turiel and Martin-Esteban, 2004). However, it should be noted that the water is to weaken the non-covalent interactions (hydrogen bond) between a template and functional monomer and hence water soluble

template molecules and monomers would also be lost due to partitioning into the aqueous phase. Furthermore, a stabilizer or surfactant, which is required for the formation and stabilization of droplet, could interfere with interactions between a template molecule and functional monomer (Jun, 2008).

2.6.3 Precipitation polymerization

With regards to precipitation polymerization, the basic principle of the approach is when the polymeric chains growing in solution reach a certain critical mass, they precipitate from the solution. The particles thus obtained do not typically exceed 10 µm and are sometimes even in the sub- µm range (Cacho et al., 2009). A crucial difference between the bulk polymerization and precipitation polymerization techniques is the volume of a polymerization medium used. The latter requires larger volumes of the medium than the former. The excess of a polymerization medium may hamper interactions between a template molecule and functional monomer (Haginaka, 2008).

2.6.4 Multi-step swelling Polymerization

A different approach to obtain spherical particles is multi-step swelling polymerization. In this technique, preformed uniformly-sized seed particles are suspended in water and the initial particles swell after several additions of suitable organic solvents. Once the particles have swollen to the desired size, all the components involved in the production of the MIP are added to the solution and incorporated into the particles in this swelling state and polymerization is then induced (Beltran *et al.*, 2010).

2.6.5 Surface Imprinting Polymerization

Surface grafting is another polymerization technique aimed at delivering spherical particles, however it is also used to produce composites materials (Tamayo and Martin-Esteban, 2005). In this case, the starting material comprises silica particles, and all the components involved in the polymerization process are adsorbed within these particles before the polymerization process starts. Once the polymer is formed, the silica is imprinted away to reveal a final product of spherical particles (Yilmaz *et al.*, 2007).

2.7 Sol Gel Process

Sol gel process provides a relatively easy way of preparing inorganic or organic-inorganic hybrid glasses through the hydrolysis and condensation of suitable metal alkoxides (Collinson, 1999). Sol gel process can be described as a creation of an oxide network by progressive polycondensation reactions of molecular precursors in a liquid medium. Generally, it starts with alcoholic or other low molecular weight organic solutions of monomeric, metal or semimetal alkoxide precursors $M(OR)_n$, where M represents a network-forming element such as Si, Ti, Zr, Al, B, etc., and R is typically an alkyl group (C_xH_{2x+1}) and water. Generally, both the hydrolysis and condensation reactions occur simultaneously once the hydrolysis reaction has been initiated (Diaz-Garcia and Lainno, 2005).

The most widely used precursors to prepare materials for use in chemical analysis applications have been the silicon alkoxides, particularly tetramethoxysilane (TMOS) or tetraethoxysilane (TEOS). These reagents can be readily hydrolyzed and condensed under relatively mild conditions as shown in the simplied reaction below (Hench and West, 1990a; Avnir *et al.,* 1994):

Hydrolysis:

$$Si(OR)_4 + nH_2O \rightarrow Si(OR)_{4-n}(OH)_n + nROH \qquad (1)$$

Condensation:

$$\equiv Si - OH + HO - Si \equiv \rightarrow \equiv Si - O - Si \equiv + H_2O \qquad (2)$$

(water condensation)

and/or

$$\equiv Si - OR + HO - Si \equiv \rightarrow \equiv Si - O - Si \equiv + ROH \qquad (3)$$

(alcohol condensation)

During the sol-gel transformation, the viscosity of the solution gradually increases as the sol becomes interconnected to form a rigid, porous network- the gel. Gelation can take place on the time scale of seconds to minutes to days to months (or longer) depending on the sol-gel processing conditions. After drying, a xerogel is formed. A large amount of shrinkage accompanies drying, often with cracking unless the monolithic materials are dried slowly or drying control addivites added to the sol (Hench and West, 1990).

The chemical reactions that occur during the formation of the sol, gel, and xerogel strongly influence the composition and properties of the final product. The physical properties (i.e., average pore size, pore size distribution, pore shape, surface area) of the dried gel depend on the sol gel process parameters and the method at which

the material is prepared (Hench and West, 1990a ; Corriu and Leclerq, 1996 ; Buckley and Greenblatt,1994 ; Osterholtz and Pohl, 1992).

2.8 Factors affecting sol-gel structure

Generally, there are four basic components in a sol which is precursor, water, alocohol and catalyst. The ratios and types of these components can affect the ultimate structure and properties of the sol-gel such as porosity, surface area, and surface functionality. Also, physical reaction conditions like pressure and temperature during sol gel processing may cause modifications in the properties of the sol-gel.

2.8.1 Basic sol-gel components: precursor, water, and alcohol

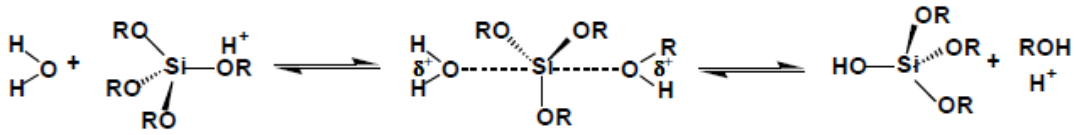
Precursors for sol-gel syntheses can be either inorganic salts or organic compounds. However, precursors are commonly metal alkoxides, predominantly silicates. Also, metal alkoxides of Ti, V, Cr, Mo, and W have been synthesized. Precursors with larger alkoxy groups lead to reaction to be slow due to increasing of steric hindrances and overcrowding of the transition state. For instance, the hydrolysis rate of TMOS is faster than that of TEOS (Wright and Sommerdijk, 2001). Also, the bulkier the alkoxy groups, the larger the average pore diameter in the final sol-gel product and slower rate constants are observed during the sol gel process (Wright and Sommerdijk, 2001; Hench and Vasconcelos, 1990b). The nature of the precursor, whether hydrophobic or hydrophilic, determines if a co-solvent is needed to achieve miscibility in water. For instance, TEOS and water are immiscible, so a co-solvent like alcohol is needed to facilitate hydrolysis. Alcohols are perhaps the most common choice for a co-solvent. However, it does not necessarily need to be included if the gel is sonicated during processing (Wright and Sommerdijk, 2001).

2.8.2 Catalyst type and concentration

The type of catalyst and its concentration can influence the structure and properties of the gel. Gels are usually catalyzed by an acid or base. Both acid and base catalyzed gels undergo a bimolecular nucleophilic substitution, S_{N2}, reaction, as shown in Figure 2.8 (Brinker and Scherer, 1990). The first step in an acid catalyzed hydrolysis reaction is the rapid protonated of an alkoxide group, causing electron density withdrawal from silicon. As a result, it is more electrophilic and is attacked by water, forming a pentacoordinate intermediate. The attack reduces the positive charge on the protonated alkoxide, making alcohol a good leaving group. The transition state decays in the final step involving the removal of alcohol and the formation of the silica tetrahedron. In the first step of a base catalyzed reaction, water dissociates to produce nucleophilic hydroxide anions. A pentacoordinated intermediate state forms. The hydroxyl anion attacks the silicon atom, displacing the alkoxide group with the inversion of the silicon tetrahedron (Hench and Vasconcelos, 1990b ; Rao and Dave, 1998).

Like hydrolysis, condensation reactions can be catalyzed by acid or by base. The condensation mechanisms are shown in Figure 2.9 (Brinker and Scherer, 1990). Both reactions proceed via the rapid formation of a charged intermediate involving a proton or hydroxide ion. The following step is slower, where the intermediate is attacked by another neutral silicon species (Brinker and Scherer, 1990).

Acid catalyst



Base catalyst

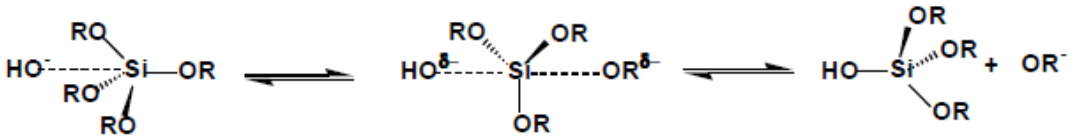
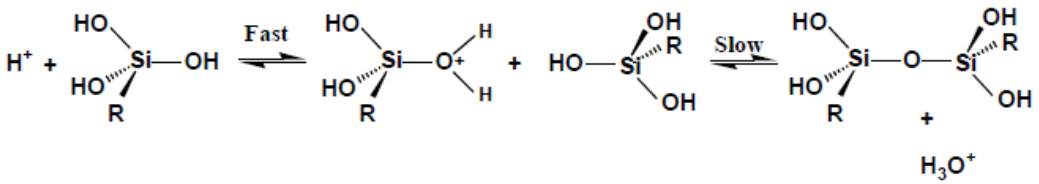


Figure 2.8: Hydrolysis mechanism (Brinker and Scherer, 1990)

The water to silane ratio, the nature and concentration of the catalyst and the alkoxide precursors strongly influence the relative rates of hydrolysis and condensation (Hench and West, 1990a; Corriu and Leclerq, 1996; Buckley and Greenblatt, 1994; Osterholtz and Pohl, 1992). Generally, at low pH and low water preparations lead to denser materials with smaller average pore sizes whereas high pH, high water preparations lead to more porous materials. Condensation process occurs between silanol groups located on monomers or the ends of polymers under acid catalysis. Under basic conditions, condensation preferentially occurs between the more highly branched oligomers to form more particulate gels. After drying, materials with high interstitial porosity are produced (Hench and West, 1990a; Buckley and Greenblatt, 1994).

Acid catalyst



Base catalyst

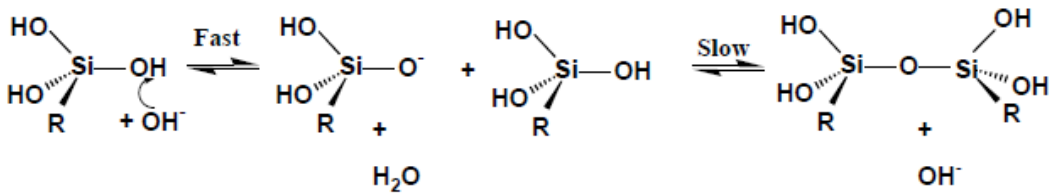


Figure 2.9 : Condensation mechanism (Brinker and Scherer, 1990)

2.9 2−Hydroxybenzoic acid (2−HA)

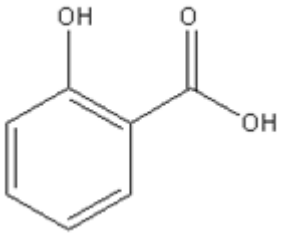


Figure 2.10: 2−Hydroxybenzoic acid

2−Hydroxybenzoic acid (2−HA), also known as salicylic acid, is an important chemical widely used as an intermediate in pharmaceuticals and cosmetics and it is introduced into the environment by a variety of industrial and natural sources (Guinea *et al.*, 2008). The molecular structure of 2-HA is shown in Figure 2.10. 2−HA can act as a kind of cosmetic in low concentration while it will bring serious environmental problems at a high concentration (Huang *et al.*, 2011). Moreover, biological degradation of salicylic acid is not feasible due to the electron-withdrawing carboxyl group on the benzene ring (Khenniche and Aissani, 2009). This aromatic organic compound can be a

toxic pollutant and maybe harmful to consumers due to their tendency to induce allergic contact dermatitis (Mikami *et al.*, 2002).

2.10 MIP for the removal of 2-Hydroxybenzoic acid (2−HA)

Zhang and their research group (2001) have studied the preparation of MIPs for several drugs having different kinds of functionalities and the mechanisms of molecular recognition. In that study, three hydroxybenzoic acids having the same functional groups, 4−hydroxybenzoic acid (4−HA), gentisic acid (GA) and 2−HA were chosen as templates in order to investigate the influence of intramolecular hydrogen bond of the template on molecular recognition using acrylamide as functional monomer. The results showed that GA and 2−HA can form intramolecular hydrogen bonds between the functional groups –OH and –COOH while 4−HA cannot form intramolecular hydrogen bond. It was proved that the hydrogen bond interaction between the functional monomer and the template played a major role in the recognition process. It was concluded that the molecular recognition ability will decrease when the template itself is able to form intramolecular hydrogen bond in the molecular imprinting.

In the following year, Zhang and co-workers (2002) had investigated the molecular imprinting behavior of the 2-HA-imprinted polymer using 4−vinylpyridine (P1) and 2-HA-imprinted polymer using acrylamide (P2) as the functional monomers respectively. No molecular imprinting effect was observed for P2 due to absence of a sufficiently stable 2-HA-acrylamide complex during the imprinting process. A significant molecular imprinting effect was, however, observed for P1 because the 2−HA−4−Vinylpyridine complex is sufficiently stable to produce selective binding sites in P1. These results proved that it is a prerequisite that the template should form a stable complex with the functional monomer in the MIP preparation process if an MIP with high molecular recognition ability is to be obtained.

In 2003, Huang and co-workers (2003b) studied the preparation of nitrophenol isomers (2−nitrophenol, 3−nitrophenol, 4−nitrophenol) and hydroxybenzoic isomers (4−hydroxybenzoic acid, 2−hydroxybenzoic acid) imprinted polymers using 4−vinylpyridine as a functional monomer in order to investigate the effect of structure and acidity of template molecules on imprinting. The results showed strong recognition ability of the resultant polymer for 2−hydroxybenzoic acid with 4−vinylpyridine as functional monomer. It seems that the structure and acidity of template molecules is responsible for the difference in recognition and influenced by the formation and strength of the interaction between template molecule and functional monomer in the imprinting process. This indicated that the imprinting of hydroxybenzoic acid isomers possessed stronger acidity than nitrophenol.

Molecular imprinting polymers for 2−hydroxybenzoic acid, 3−hydroxybenzoic acid (3-HA) and 4−HA were prepared using styrene and 4−vinylpyridine and divinylbenzene as crosslinker by Park and his research group (Park *et al.,* 2007). 3−HA and 4−HA adsorb very well however 2−HA−MIP had no molecular imprinting due to 2−HA has intramolecular hydrogen bond and it is difficult to adsorb on recognition site of 2−HA−MIP. 2-HA can be separated selectively when using 1,2,3,4−tetrahydro−1−napthol.

In 2008, Li and his research group (Li *et al.,* 2008) had improved the preparation of MIP by using emulsion polymerization technique where this polymerization technique is rarely reported. In their study, spherical MIPs with specific recognition to 2−HA−MIP were synthesized by polymerization in oil-in-water emulsions with 2−HA as a template and acrylamide as a functional monomer. The interaction between the template and functional monomer was confirmed by UV spectra in which the wavelength corresponds to the adsorption peak at the lower wave band with increasing

of acrylamide/2−HA molar ratio. This indicated that hydrogen bonds and ionic interaction exist between 2−HA and acrylamide.

Recently, molecularly imprinted sol gel materials (MISG) have been extensively studied because they have been verified to be much more specific toward the target species compared to the traditional imprinted method. The sol gel process combined with surface imprinting technology has been proved efficient and well developed for specific adsorption of guest target (Fang *et al.*, 2005; Han *et al.*, 2005). Due to this reason, MISG method was highlighted in this research in order to enhance the affinity and selectivity.

CHAPTER 3

METHODOLOGY

3.1 Chemical Reagents

Chemicals are listed in Tables 3.1. HPLC grade solvents and the reagents were used without further purification.

Table 3.1: List of reagents used

Chemical	Supplier	Assay (%)
2−Hydroxybenzoic Acid (2-HA)	Fisher Scientific	100.07
3−Hydroxybenzoic Acid (3-HA)	Merck	≥98
4−Hydroxybenzoic Acid (4-HA)	Merck	≥98
Benzoic acid	Merck	≥98
Phenol	Merck	≥99
3−Aminopropyltriethoxysilane (APTES)	Sigma Aldrich	≥98
Tetraethoxysilane (TEOS)	Sigma-Aldrich	98
Acetic acid (HAc)	Sigma-Aldrich	99
Hydrochloric acid (HCl)	Merck	37
Natrium hydroxide (NaOH)	R&M Chemicals	99
Ethanol (EtOH)	RCI Labscan	99.9
Acetonitrile	RCI Labscan	99.9
Silica gel (60-200 mesh)	Sigma Aldrich	-

3.2 Instrumentation

3.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectra were recorded on a Perkin–Elmer RX1 FT-IR spectrometer with samples prepared as KBr pellets. All spectra were run in the range of 400–4000 cm^{-1} at room temperature.

3.2.2 Scanning Electron Microscopy

The morphology of the polymers was characterized by SEM (Model No. LEO 1450 VPSEM, United Kingdom). The microcapsules were attached to stub using 2-sided adhesive tape. Meanwhile, the specimens were coated with gold–palladium (Plasma deposition method), with BIO-RAD AC500, and examined at 20 kV. The surface of the polymer samples was then scanned at the desired magnification to study the morphology of the particles.

3.2.3 Surface Area and Porosity Analysis

BET method, whereby physical adsorption of gas molecules on a solid surface and serves as the basic for an important analytical technique for the measurement of the specific surface area of a material. The analysis of BET surface area measurements were obtained by performing nitrogen adsorption at liquid nitrogen temperature (77 K). Typically, at least 0.5000 g sample was used each time.

3.2.4 Thermal Gravity Analysis (TGA)

Thermogravimetric analyses (TGA) is a technique in which the mass of a substance is measured as a function of temperature, while the substance is subjected to a controlled temperature programme. Measurements are used primarily to determine the composition of materials and to predict their thermal stability at temperatures up to 1000 °C. The technique can characterize materials that exhibit weight loss or gain due

to decomposition, oxidation, or dehydration. TGA were performed using a TA Instruments Q500. Samples were heated at 20 °C/min from room temperature to 900 °C in a dynamic nitrogen atmosphere. About 3-10 mg of 2-HA-MISG and NISG samples were filled in alumina crucible and heated under nitrogen flow.

3.2.5 Ultraviolet-Visible (UV-vis) Spectroscopy

Spectrophotometric measurements were made with a Shimadzu UV 265 UV–vis recording spectrophotometer using 1 cm quartz cells. Daily checked was done in order to compare all spectrophotometric measurements and to ensure reproducible experimental conditions, of UV-265 spectrophotometer.

3.2.6 High Performance Liquid Chromatography (HPLC) analysis

HPLC-UV analysis was performed using a chromatographic system, CBM-20A communication bus module from Shimadzu, Japan. HPLC-UV system consisted of a LC-20AT pump, a SPD-M20A diode array detector, a SIL-20AHT auto sampler and a CTO-20AC column oven. All separations were achieved on an analytical reversed-phase Chromolith RP-18 monolithic column (100 mm x 4.6 mm i.d., Merck, Germany) under isocratic conditions at a column temperature 35 °C with a mobile phase containing acetonitrile/acid in water (50:50, v/v) which is formic acid (0.1 M), at a flow rate of 0.8 mL/min . The injection value was 20 µL and detection was accomplished at 204 nm.

3.3 Calibration Curve

A series of standard dilution were prepared in deionized water over the range of 2 ppm to 10 ppm. Five concentrations of standard were injected to the HPLC system. Calibration curve was calculated applying the least-square method to concentration (ppm) versus peak area ($\times 10^5$). The calibration curve obtained was linear (correlation coefficient, $R^2 > 0.99$).

3.4 Preparation of 2-HA- molecularly imprinted silica gel sorbent (2-HA-MISG)

2-HA-MISG was prepared by the surface molecular imprinting technique with a sol gel method based on method proposed by Han *et al.*, 2010a. Silica gel was heated overnight in order to activate the surface. To prepare the 2-HA imprinted amino-functionalized silica gel sorbent, 1 g of 2−HA was dissolved in 5 mL of ethanol and mixed with 2 mL of APTES. The mixture was stirred for 20 min, and then 4 mL of TEOS was added. After stirring for 5 minutes, 1 g of activated silica gel and 1 mL of 1M HAc were added. The mixture began to co-hydrolyzed and co-condensed after stirring for a few minutes, then incubated overnight in a water bath. The product was filtered and dried in a vacuum oven at 100 °C for 8 hours. Thus, the activated silica gel surface was grafted with the complex. For comparison, the non-imprinted silica gel sorbent (NISG) was also prepared using an identical procedure, but without the addition of 2−HA. The 2-HA loaded silica gel (2-HA-SG) was extracted with ethanol and 6 M HCl under stirring for 2 hours to remove 2−HA. The product was isolated by filtration, washed with the mixture of ethanol and 1 M HCl, neutralized with 0.1 M NaOH, and then washed with pure water. This procedure was repeated several times until no template molecules were detected or in very low concentration. Finally, the sorbent was dried in oven at 80 °C for 12 hours.

3.5 Static and kinetic adsorption tests of 2-HA onto 2-HA-MISG

3.5.1 Effect of pH

To test the effect of pH, 20 mg of 2−HA−MISG sorbents were equilibrated with 10 mL solutions containing 10 ppm of 2−HA and the pH were adjusted by adding either HCl or NaOH. The mixtures were mechanically shaken for 1 hr at room temperature and separated by centrifugation. The filtrate was measured for the unextracted 2−HA by HPLC.

3.5.2 Effect of contact time

The kinetics on the sorption of 2−HA was studied from 5 to 60 min. 20 mg of 2−HA−MISG was added to 10 mL of 10 ppm of 2−HA solution. The mixture was mechanically shaken and filtered and then analyzed using HPLC.

3.5.3 Effect of initial concentration

To measure the adsorption capacity, 20 mg of 2−HA−MISG was mixed with different concentration of 2-HA (20 ppm – 400 ppm) and shaken for 1 hr. The filtrate was measured by UV-Vis spectrophotometer.

3.5.4 Determination of adsorption capacity

To calculate the adsorption capacity (mg/g) of 2−HA−MISG for sorption of 2−HA, the binding capacity at equilibrium time (Q_t) will be determined respectively according to the following equations (Han *et al.*, 2010a):

$$Q_t = (C_0 - C_t)\, V/\, W \qquad (4)$$

where, C_0 = the initial concentration of 2−HA (mg/L)
C_t = the equilibrium concentration of 2−HA (mg/L)
V = the volume of 2−HA solution (L)
W = the mass of 2−HA−MISG (g)

3.6 Selectivity study

Selectivity studies were performed by 2−HA−MISG and NISG containing 3−HA, 4−HA and phenol solution as comparative agents since the molecular structures of the chemicals are similar to 2−HA to a certain extent. The 2−HA−MISG (20 mg) and NISG sorbent were added to 10 mL of 10 ppm of 2−HA, 3−HA, 4−HA, benzoic acid and phenol solution respectively and shaken at room temperature for 1 hr, and then separated centrifugally. HPLC was used to measure the unextracted target molecules.

The distribution constant (K_d, L/g) for each substance was calculated using Eq. (5):

$$K_d = \frac{Q}{C_e} \qquad (5)$$

where Q (mg/g) and C_e (mg/L) are as described previously.

The selectivity coefficient (k) of MISG for 2−HA with respect to the analogues (3−HA, 4−HA, phenol), referred to as B in Eq. (6) was calculated from the distribution constant as indicated in Eq. (5):

$$k = \frac{K_{d,(2-HA)}}{K_{d,B}} \quad (6)$$

The value of k gives an indication on the recognition ability and selectiveness of the MISG for 2−HA with respect to other similar compounds. Similarly, a relative selectivity coefficient k' can be calculated as illustrated in Eq. (7), where the value of k' shows the imprinting effect on binding affinity and selectivity of MISG for 2−HA over NISG.

$$k' = \frac{K_{MISG}}{K_{NISG}} \quad (7)$$

3.7 Reusability of 2-HA-MISG

The imprinted functionalized sorbent was used to extract 2−HA through regeneration process to test of sorbent recyclability. The mixture solution of ethanol and 6 M HCl (V:V = 1:1) was used to strip the adsorbed 2−HA, then the material was filtered and neutralized with 0.1 M NaOH and washed with pure water. The 20 mg of regenerated 2−HA−MISG was mixed with 2−HA solution and shaken for 1 hour. The filtrate was then analyzed using HPLC.

3.8 Application to real samples

1 g of the cosmetic product (different brand of facial wash namely 1, 2, 3, and 4 respectively) was accurately weighed in a 10 mL volumetric flask and diluted with ethanol and the cosmetic samples were spiked with an appropriate amount of 2−HA solution with the addition of 20 mg of sorbent. The mixtures were mechanically shaken for 60 minutes at room temperature and then centrifugally separated. The filtrate was measured for the unextracted 2−HA by HPLC.

CHAPTER 4

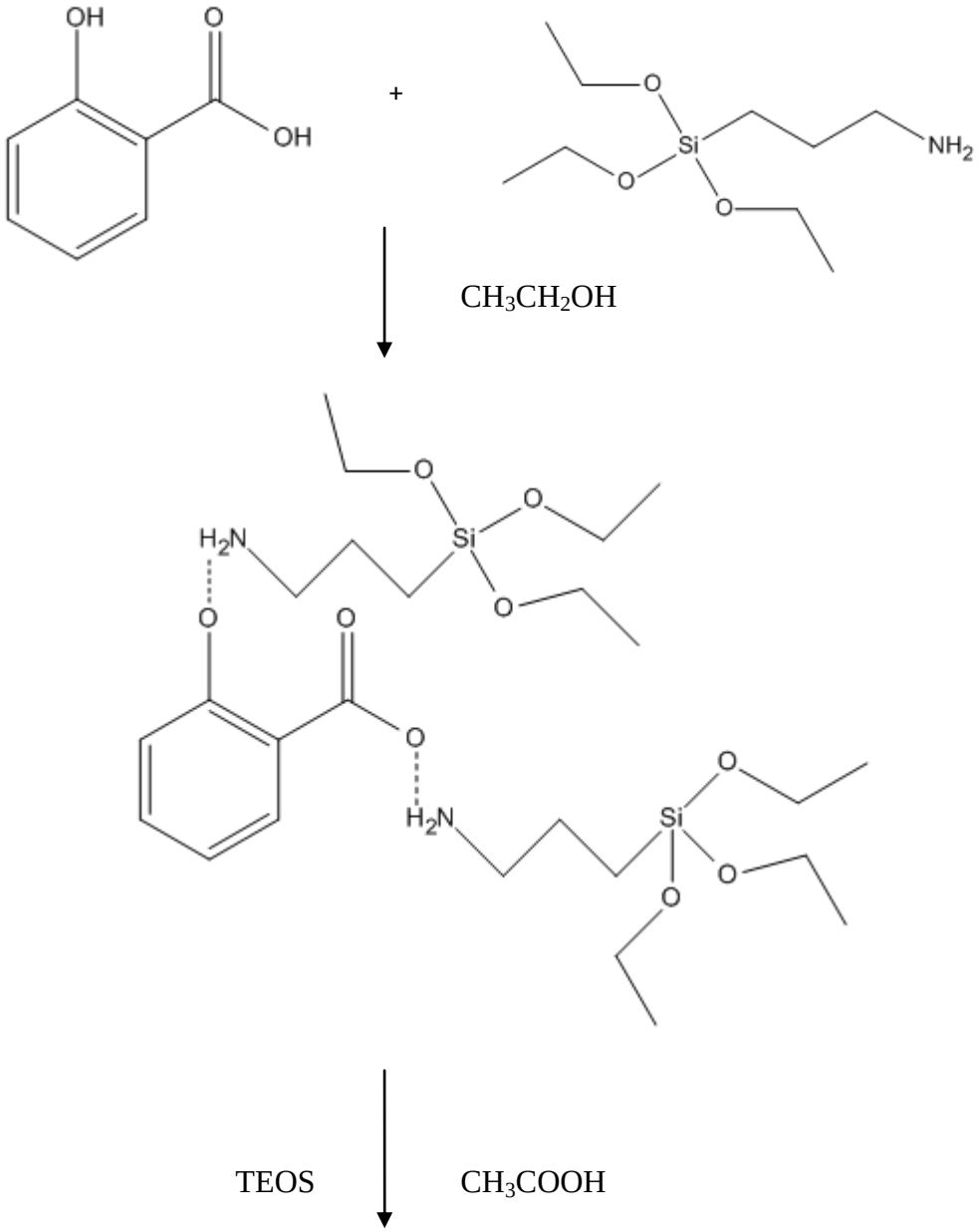
RESULTS AND DISCUSSION

4.1 Preparation of 2−HA−MISG

2−HA molecularly imprinted silica gel sorbent was prepared using 2−HA as template, APTES as a functional monomer and TEOS as a crosslinker dissolved in small amount of ethanol. Then, activated silica gel and 1M HAc were added to the mixture solution. The ratio was used based on previous study by Han and his research group (Han *et al*., 2010a).

The principle of molecular imprinting lies in the preservation of the pre-polymerized host/guest structure into a polymeric matrix. Thus, it is important that the template and the functional monomer can form stable complexes through hydrogen bonding, ionic bonding or other interaction forces in the pre-polymerization mixture (Fan *et al.*, 2009). Figure 4.1 shows the possible reaction mechanism of 2−HA−MISG preparation. In this procedure, APTES was used as a functional monomer and combined with 2−HA mainly by carboxyl groups and hydroxyl groups. Complexation between APTES and 2−HA would form strong hydrogen bonding between amino group of APTES with hydroxyl and carboxyl group of 2−HA (Yin *et al.*, 2012). TEOS acts as a cross-linker agent to maintain the stability of the recognition sites (Yan and Row, 2006) under the existence of HAc catalyst. HAc is often used as an activator to accelerate the

formation of the polymer (Zhu *et al.*, 2011). Meanwhile, HCl was used to break 2−HA/APTES bond for removal 2-HA in the polymer (He *et al.*, 2008).



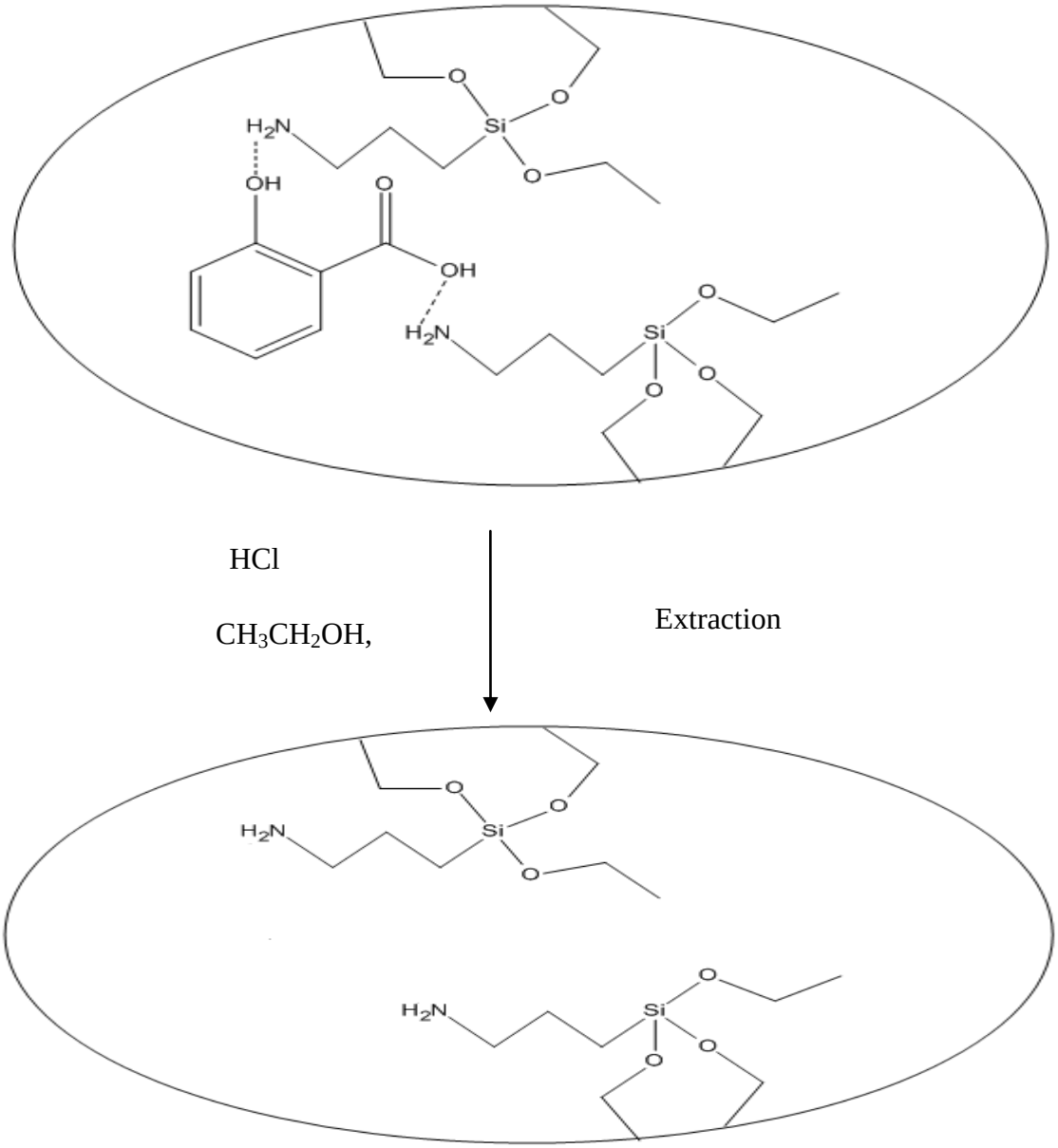


Figure 4.1: Schematic mechanism of 2−HA−MISG preparation.

4.2 Characterization of 2−HA−MISG

4.2.1 IR Spectra

FTIR spectroscopy was employed for activated silica gel, 2−HA−SG and NISG sorbents are compared in Figure 4.2. The IR spectrum of activated SiO_2 showed that the bands around 1096.31 cm^{-1} and 974.62 cm^{-1} indicated Si−O−Si vibration and Si−O−H stretching vibrations, respectively. And the peaks around 803.66 cm^{-1} and 467.22 cm^{-1} were attributed to Si−O vibrations in the form of silica particles respectively.

The strong intensity of the 2−HA−SG bands at 1448 cm^{-1}, 1459 cm^{-1} and 1388 cm^{-1} represent C = C bonds of aromatic ring from 2−HA. Also, conjugation of C=O with C=C from 2−HA lowered stretching frequency to 1627 cm^{-1}. This suggested that the template molecule was successfully embedded within the 2−HA−SG.

Imprinted and non-imprinted sorbents showed similar locations and appearances of the major bands. The FTIR spectrum for the 2−HA−SG and NISG showed a broad absorption bands at 3445 cm^{-1} and 3448 cm^{-1} respectively, which can be attributed to O−H stretching vibration of OH bonded to the adsorbent surface. The absorptions around 1594 cm^{-1} for 2-HA-SG and 1555 cm^{-1} for NISG indicated -NH_2 had been grafted onto the surface of activated silica gel after modification, so the APTES had combined with the surface of the functionalized silica gel sorbent.

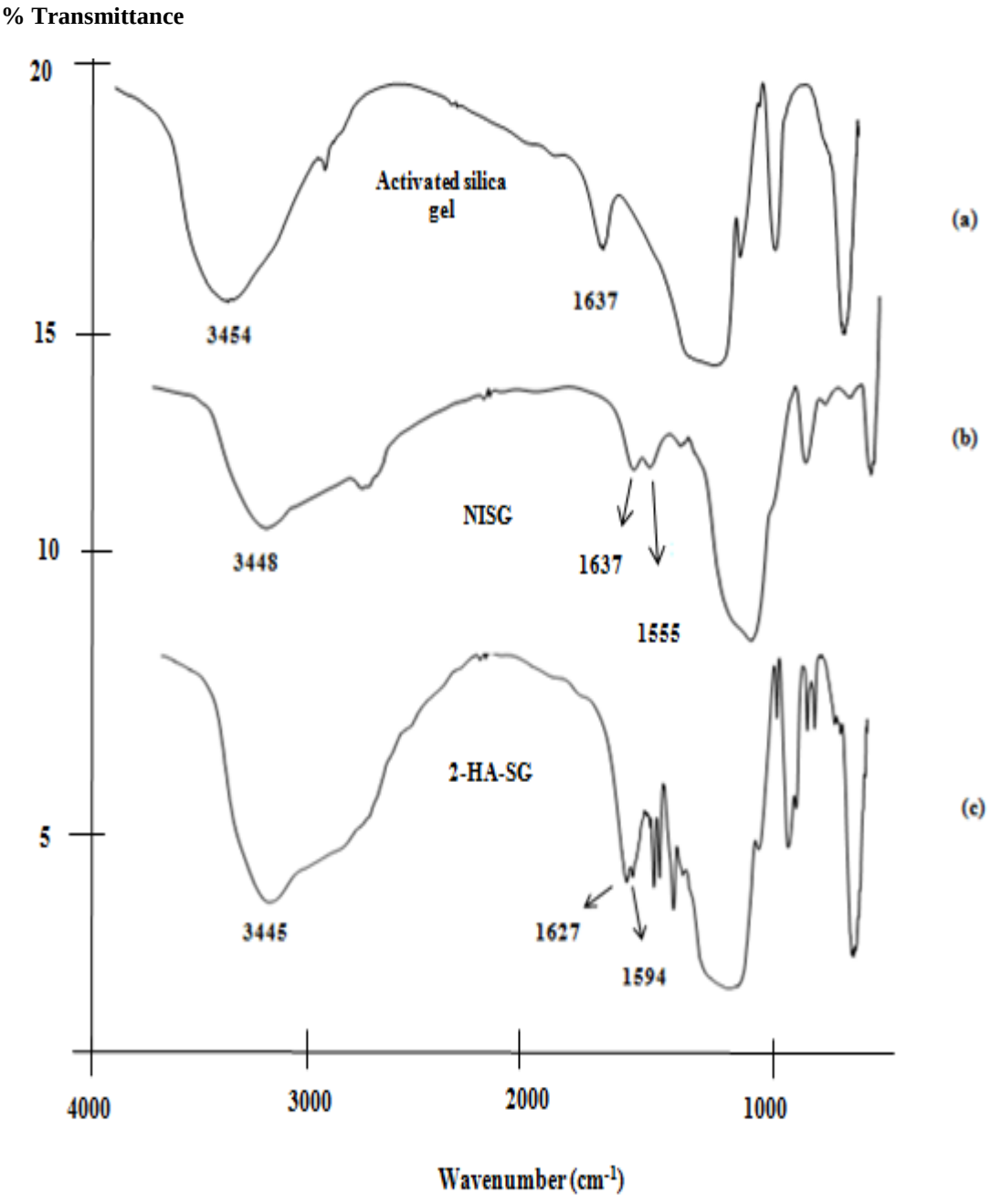


Figure 4.2: IR spectra of activated silica gel (a), NISG (b) and 2-HA-SG (c)

4.2.2 Thermogravimetric (TGA) analysis of 2−HA−SG

TGA analysis provides an important tool for thermal stability study and this technique allows the determination of the temperature range at which a heated sample undergoes a major conformational change by means of monitoring the thermal weight loss profile (Soares *et al.,* 2004). The thermogram was used in order to confirm the presence of the template in MISG and to investigate the differences of decomposition stage of 2−HA−SG and NISG particles.

Figures 4.3 illustrates thermogravimetric curve for (a) NISG and (b) 2−HA−SG particle respectively. As shown in Figure 4.3 (a), NISG is totally decomposed at 140.59 °C. The decomposition of NISG is fast compared to 2−HA−SG due to the absence of 2−HA which acts as the template.

Figure 4.3 (b) shows that at temperatures from 50 °C to 193.01 °C, the weight loss was mainly due to the loss of absorbed water and the curve from 193.01 °C to 361.80 °C can be attributed to the breaking of 2−HA on the surface of silica. The weight loss increased rapidly from 361.80 °C to 800 °C because of the thermal decomposition of the polymer. Beyond 800 °C, the entire polymer on the surface of silica was completely decomposed. Therefore, based on the 2−HA−SG TGA plot it can be concluded that 2−HA−SG is quite stable at high temperature.

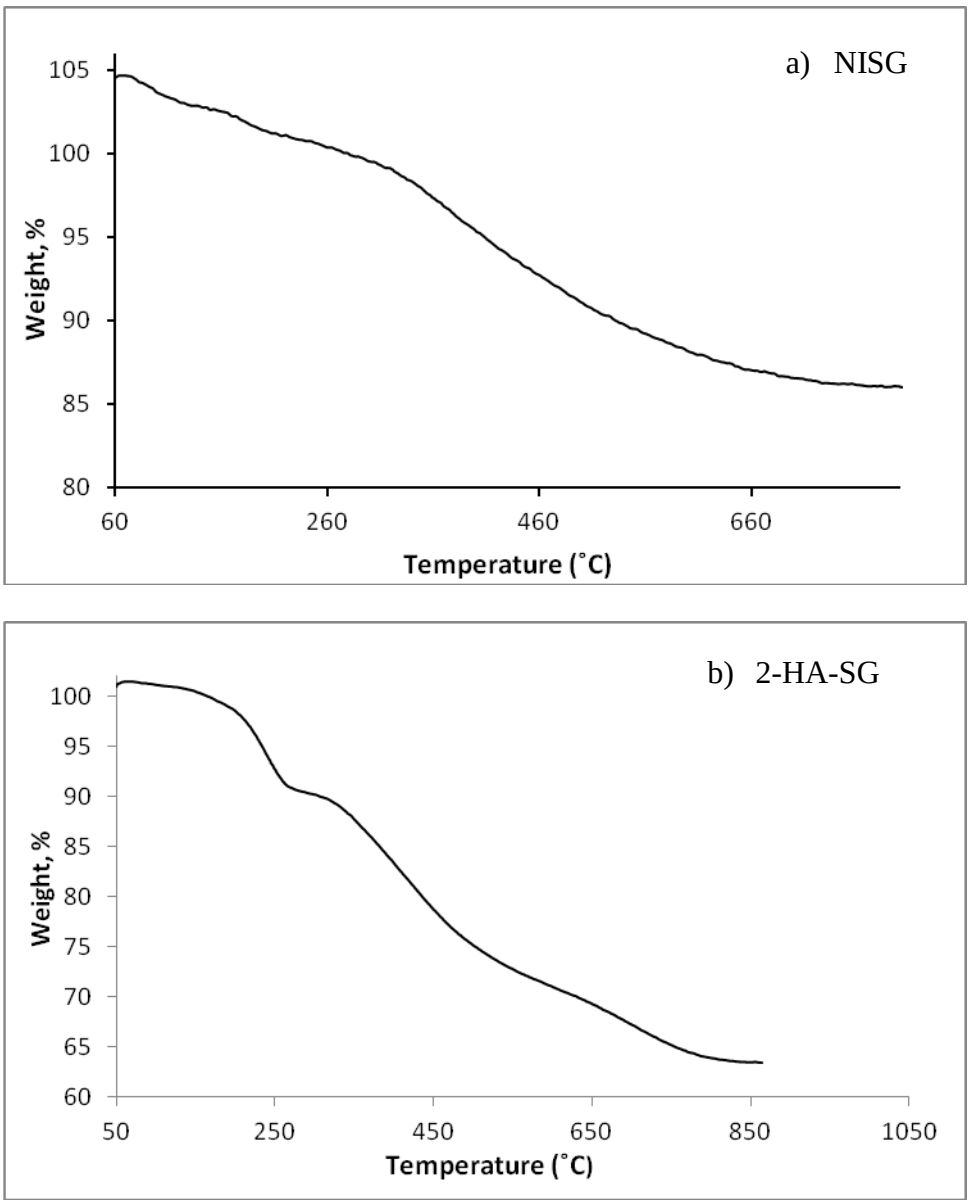
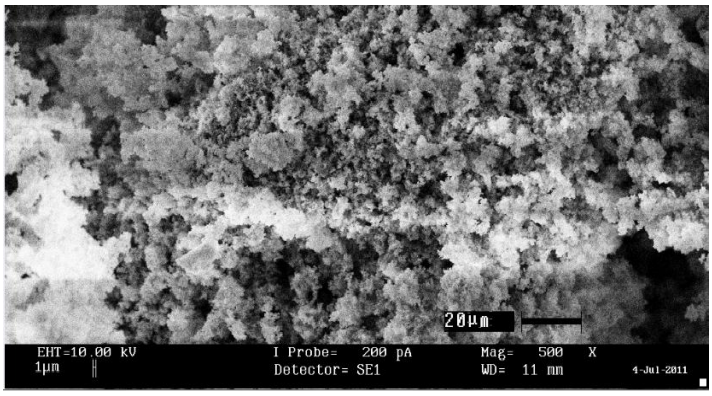


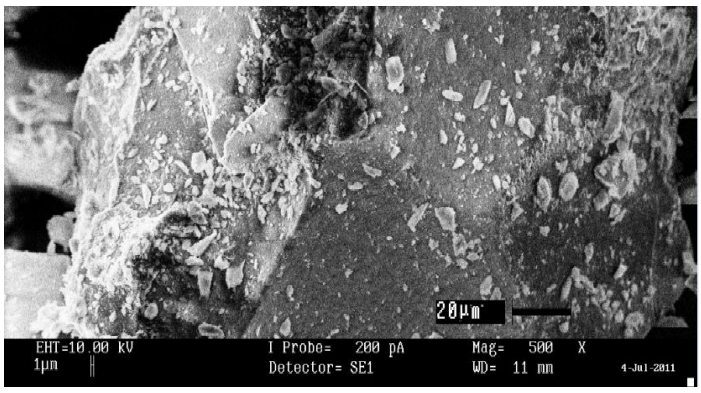
Figure 4.3: TGA curves of (a) NISG and (b) 2−HA−SG

4.2.3 Scanning electron microscopic (SEM)

The morphologies of 2−HA−MISG and NISG are shown in Figure 4.4. For both polymers, it can be seen that the agglomerates of microparticles are of different sizes. The particles possess porous surface due to pores with irregular shapes and sizes that can play an important role in the adsorption process. The roughness of the particle surface itself providing an increase in the surface area (Saliza *et al.,* 2011). However, the particles of NISG possess uniform, compact and smooth shape which had an irregular and rough morphology (Saliza *et al.,* 2011). This structure is due to the lack of specific binding sites created as in 2−HA−MISG.



(a)



(b)

Figure 4.4: SEM micrographs of (a) NISG (magnification x 500) and (b) 2-HA-SG (magnification x 500)

4.2.4 Surface area, porosity analysis and BET isotherm of 2−HA−MISG

Nitrogen sorption porosimetry is a particularly useful method to analyze in detail medium-sized (meso-) and small-sized (micro-) pores (Cormack and Elorza, 2004). Based on the IUPAC classification, the term macropores are used for pore diameters greater than 50 nm, mesopores is for the pore diameter in the range of 2 to 50 nm and micropores is for pore diameters less than 2 nm. There are 6 types of adsorption isotherms shown in Figure 4.5 which can be used to understand the concept of porosity (Sing, 1982):

Type I is the most common isotherm encountered and is characteristic of materials having extremely small pores.

Type II is for non-porous or macroporous materials. The inflection point or knee of the isotherm is called Point B. This point indicates the stage at which monolayer coverage is complete and multilayer adsorption begins.

Type III is for adsorption on non-porous or macroporous adsorbent with weak adsorbate-adsorbent interaction.

Type IV is typical of mesoporous materials. The characteristic feature of the type IV isotherm is the occurrence of hysteresis loops. Hysteresis loops arise from different adsorption/desorption mechanisms.

Type V represents pore condensation and hysteresis in mesopore range. This type is not common and the adsorbate-adsorbate interactions are weak. Meanwhile, type VI represents stepwise multilayer adsorption on a uniform and non-porous surface.

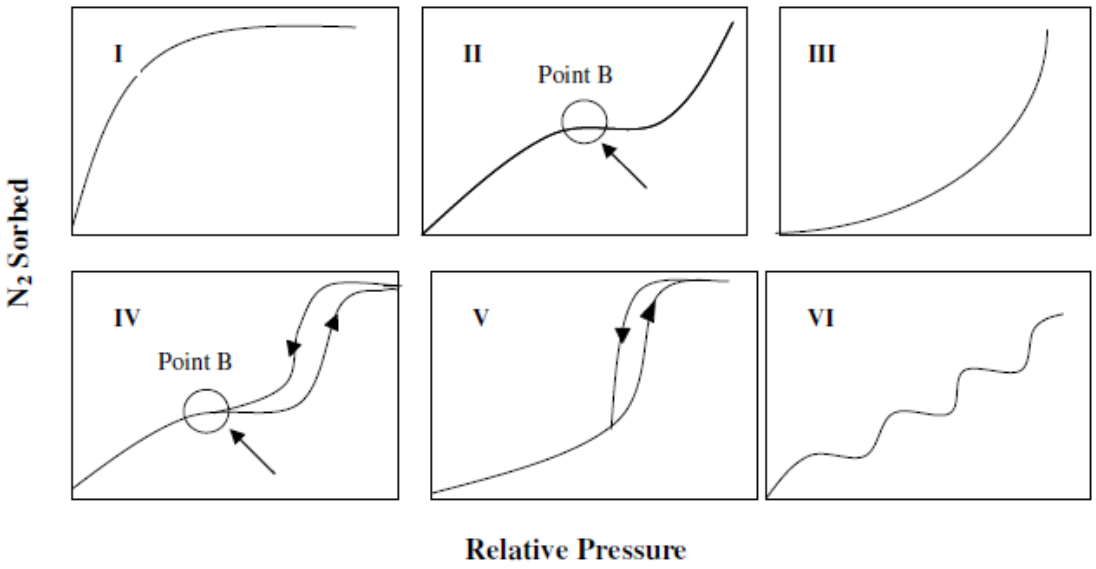


Figure 4.5: The IUPAC classification for adsorption isotherm with x-axis represent the relative pressure and the y-axis denotes the amount of gas adsorbed (Sing, 1982)

Figure 4.6 represents N_2 gas adsorption-desorption isotherm of the 2-HA-SG. It is clear that the isotherm exhibits the typical II curve according to IUPAC classification with a hysteresis loop which reflects the formation of a macroporous structure. This macroporous structure would facilitate the mass transfer of analyte in binding experiments. The volume adsorbs increases slowly at a low relative pressure in a range of 0.0-0.9, followed by a rapid adsorption at a relative pressure more than 0.9. The sharp increase and a hysteresis loop at a high relative pressure (P/P_o = 0.96601) indicating the existence of macropores were observed. This results was supported by Sing (1995) where Type II isotherms are also obtained with macroporous solids since capillary condensation occurs in macropores only at high P/P_o (at high $P/P_o > 0.95$).

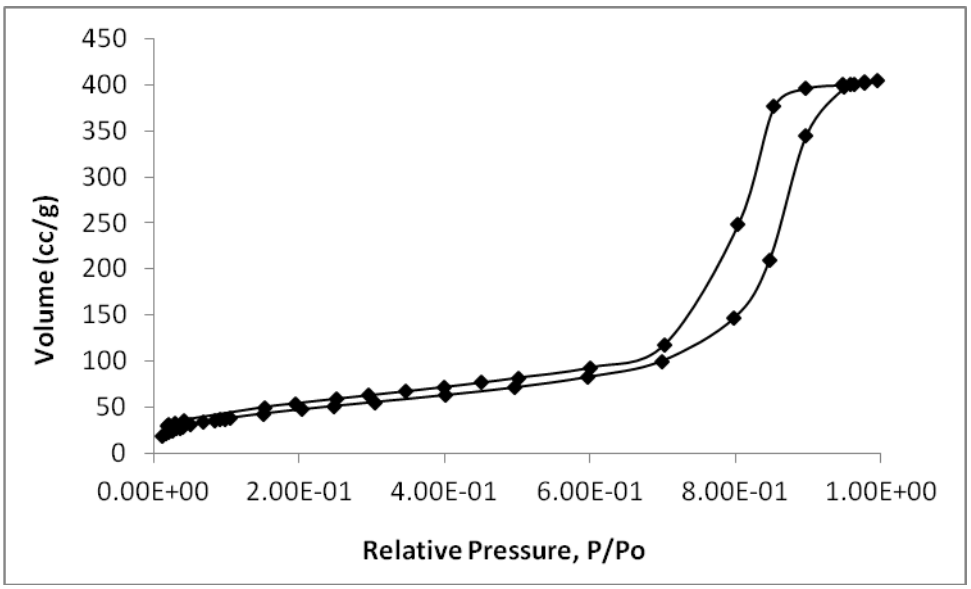


Figure 4.6: Nitrogen adsorption and desorption of 2−HA−SG

Brunauer, Emmett and Teller developed the BET method in 1938 by extending the Langmuir method to include multilayer adsorption. A BET instrument records changes in pressure as a gas, usually nitrogen is pumped into a system. The measurements are done at constant temperature (isotherm) in order to make the calculations easier. The pressure in a closed system will proportionally increase as the number of gas molecules increases. However, it is not that simple because of adsorption. Brunauer made the assumption that further physisorption is possible after the initial first layer, i.e multilayer adsorption. However, they assumed that there were no interactions between each adsorption layer. Like Langmuir, they assumed homogenous adsorption sites in which all molecules in layers other than the first have the same adsorption energy and the enthalpy of adsorption is similar for all molecules in a given layer. The BET method is useful for Types II and IV isotherms.

The BET equation is often represented as:

$$P_s/V_A(P_o-P_s) = 1/V_mC + (C-1)(P_s/P_o)/V_mC \quad (10)$$

where P_s is the sample pressure, V_A is the volume adsorbed, P_o is the saturation pressure, P_s is the sample pressure, V_m is the volume of monolayer and C is the constant related to enthalpy of adsorption (Brunauer *et al.,* 1938).

Nitrogen adsorption/desorption analysis of Brunauer-Emmett- Teller (BET) was used to evaluate the BET surface area, pore volume and pore size of the sorbent. The results were summarized in Table 4.1.

Table 4.1: Porosities of particles determined by BET analysis

Sample	NISG	2−HA−SG	2-HA-MISG (after extraction)
BET surface area (m^2g^{-1})	102.2	177.2	294.4
Pore volume (m^2g^{-1})	0.2873	0.6199	1.130
Pore size (nm)	112.5	140	153.5

It is proved that 2−HA−MISG (294.4 m^2g^{-1}) has large specific area than NISG (102.2 m^2g^{-1}). This is due to imprinting of template (2−HA) on the surface of 2−HA−MISG sorbent resulting in a particles having larger surface area (Saliza *et al.,* 2011). The 2−HA−MISG after extraction showed particle with high porosity suggesting better- defined spherical polymer macroparticles (Saliza *et al.,* 2011).

Pore size are classified in accordance with the classification adopted by the International Union of Pure and Applied Chemistry (IUPAC) [19] , that is micropores (diameter (d) < 20 Å), mesopores (20 Å < d < 500 Å), and macropores (d < 500 Å). The average pore diameter determined by Barrett-Joyner-Halenda (BJH) method was 153.5 Å suggesting that 2-HA-MISG consists of macroporous structures.

Generally, the large pore volume possesses large specific surface area. This is advantageous for the polymer to adsorb the template molecule (Fan *et al.,* 2009). It was found from Table 4.1 that the average pore volume of 2-HA-MISG (1.13 m^2g^{-1}) was larger than NISG (0.2873 m^2g^{-1}). This indicated that 2-HA-MISG has strong specific adsorption for 2−HA. These data support the observation from SEM images.

4.3 Evaluation of 2-HA adsorption by 2-HA-MISG

4.3.1 Effect of pH

The pH of the adsorbate solution has influence on the whole adsorption capacity, the surface charge and the ionization of the functional groups on the adsorbent, and the degree of ionization of the molecules in solution. H^+ and OH^- are usually strongly adsorbed and therefore, the adsorption of other ions is affected by the solution pH. Generally, anions can be easily adsorbed at low pH values due to the presence of H^+ ions on the negatively charged solid surfaces (Dai *et al.,* 2011).

As shown in Figure 4.7, the adsorption capacity of the polymer for the 2−HA reached the maximum between pH 2 and pH 4. The adsorption capacity however decreased significantly after pH 4. This could be interpreted by the ionization of 2−HA. 2−HA at low pH is almost undissociated (weak acid, pKa = 2.98). Therefore, controlling the pH of solution to match the pKa can affect the 2−HA to change its deprotonate or protonated form. The relative ability for molecule to give up a proton (deprotonate) is measured by its pKa value. In addition, if the pH values are higher than the pKa values of 2−HA this would result in mostly the deprotonate form of the compound which then lead to the decreased in removal efficiency. Under these conditions, the imprinted surfaces are generally covered with amino groups in various forms at different pH levels (Liu and Huang, 2011). As pH is becoming lower (pH 2, pH 3 and pH 4), amino groups were protonated to form the positively sites (for example

–NH_3^+ groups) and electrostatic attraction occurred between 2−HA analyte solution and –NH_3^+ leading to the increased of removal efficiency under strong acidic conditions. Meanwhile, at higher pH values, the concentration of H^+ was decreased and at the same time the concentration of OH^- was increased which will be compete with 2−HA analyte solution. Therefore, the ability of $–NH_2$ to be protonated was weakened resulting in the decline in removal efficiency (Liu and Huang, 2011). Therefore, pH 2 was chosen for the following adsorption test due to maximum adsorption capacity.

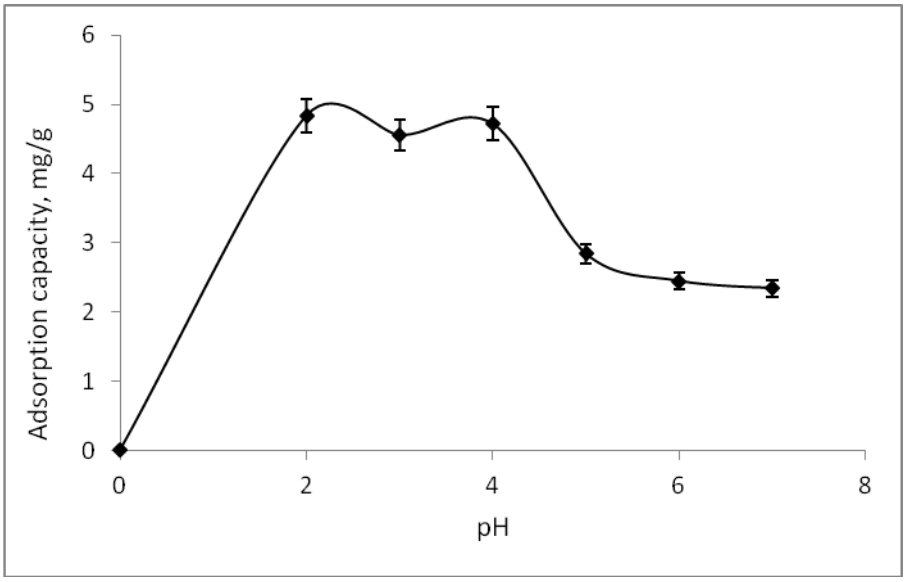


Figure 4.7: Effect of pH on 2−HA adsorption onto 2−HA−MISG

4.3.2 Effect of contact time

The kinetic profile of 2−HA−MISG was examined at a 2−HA concentration of 10 ppm and illustrated in Figure 4.8. It can be seen that the imprinted sorbent has a fast uptake kinetics within a short shaking period of 5 min, and adsorption equilibrium was achieved in 20 minutes. This was because of the fact that a large number of vacant surface sites were available for adsorption at the initial stage. The amount of adsorbate is rapidly accumulated on the adsorbent surface mainly within the 5 minutes after which the remaining vacant sites are difficult to be occupied due to the formation of

repulsive forces between 2−HA on adsorbent surface and in solution phase owing to decrease in concentration gradient. The decrease of adsorption rates is well illustrated by the plateau line after 20 minutes of adsorption (Srivastava *et al.,* 2006). This indicated that the MISG displays fast kinetics for 2−HA adsorption.

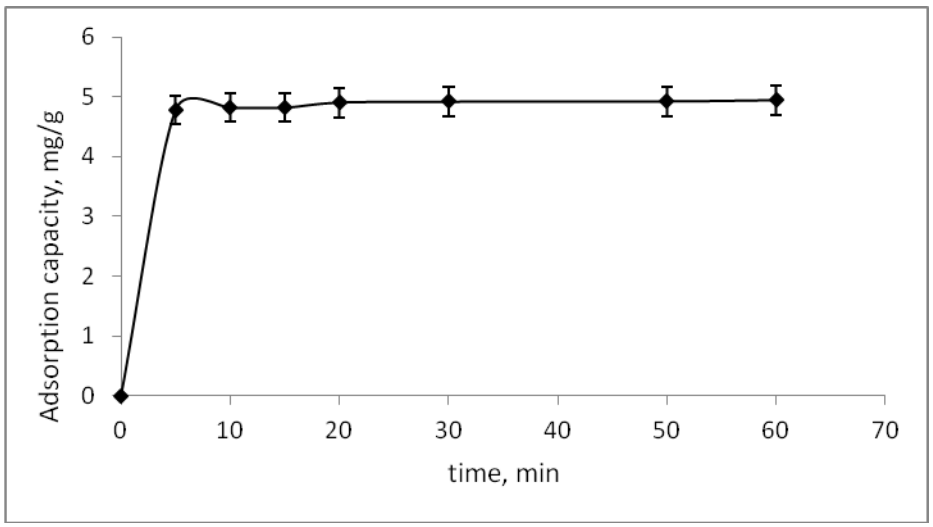


Figure 4.8: Effect of time on 2−HA adsorption onto 2−HA−MISG

4.3.3 Effect of initial concentration

Figure 4.9 shows the dependence of the initial concentration of 2−HA solution on the adsorption of 2−HA on the imprinted polymer sorbent. The adsorption capacities of 2−HA−MISG increased almost linearly with the initial concentrations of 2−HA. When the initial concentration of 2−HA solution increases from 20 ppm to 400 ppm, the adsorbed amount of 2−HA increases from 8.57 mg/g to 60.95 mg/g. The initial concentration provided the necessary driving force to overcome the resistances to the mass transfer of adsorbents between the aqueous and solid phases (Han *et al.,* 2010b). On the other hand, the percentage uptake shows a decreasing trend as the initial concentration of the 2−HA is increased. At lower concentrations, all sorbate molecule present in the sorption medium could interact with the binding sites, hence higher percentage uptake results. At higher concentrations, because of the saturation of the

sorption sites, the percentage uptake of the 2−HA by 2−HA−MISG shows a decreasing trend. The same trend was also observed by Aravindhan *et al.,* (2007).

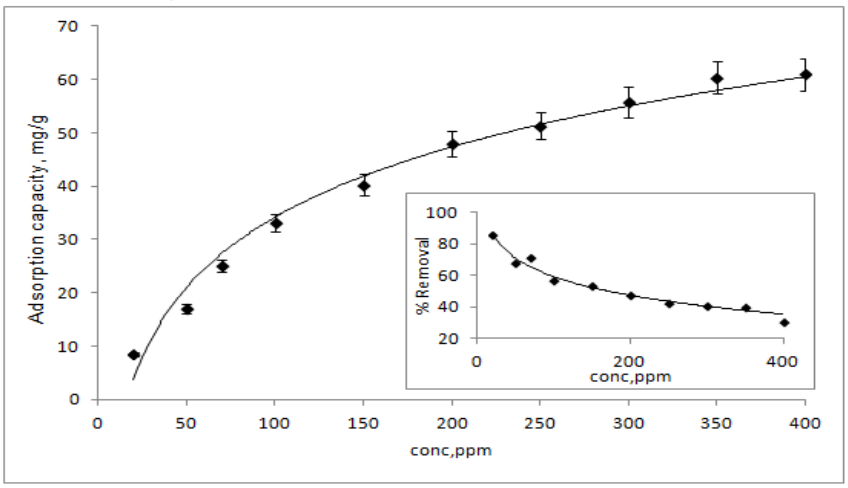


Figure 4.9: Effect of initial concentrations on 2−HA adsorption onto 2−HA−MISG

4.3.4 Binding selectivity of the sorbent

Adsorption and competitive recognition studies were performed with 2−HA and structurally related compounds 3−HA, 4−HA and phenol as shown in Figure 4.10.

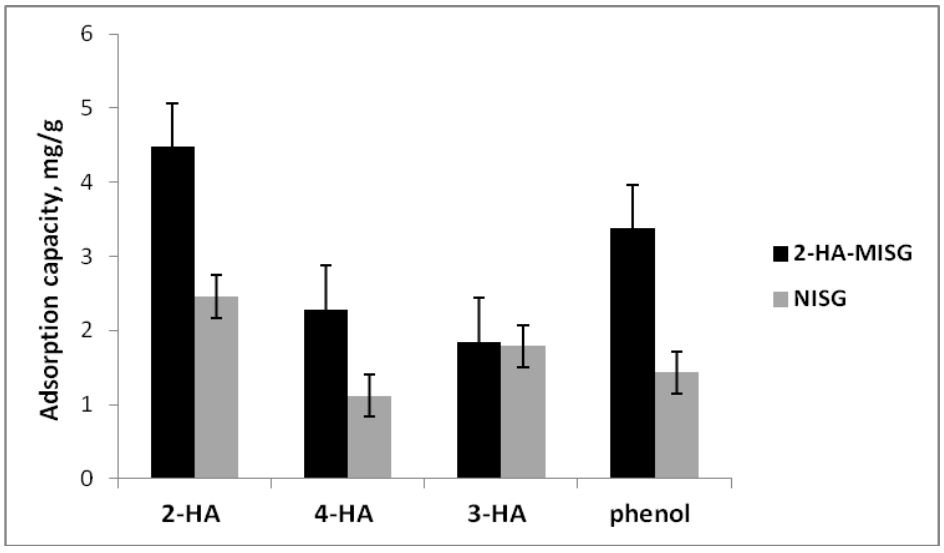


Figure 4.10: Selectivity study on 2−HA, 3−HA, 4−HA and phenol

Table 4.2 summarizes the data for adsorption capacity, distribution coefficient (K_d), selectivity coefficient of the sorbent (k) and the relative selectivity coefficient (k') obtained in these competitive binding experiments. The k values for the imprinted sorbent with the corresponding non-imprinted sorbent indicated that significant increase in k for 2−HA through imprinting. A similar result was obtained by Han *et al.,* (2010a) in their study on the selective removal of 2,4−dichlorophenoxyacetic acid by molecularly imprinted amino-functionalized silica gel sorbent. For example, the k (2−HA/3−HA) value of the imprinted sorbent (14.59) is 8.3-fold that of non-imprinted sorbent (1.75).

The large k value of the imprinted sorbent is an indicative of its high selectivity for 2−HA over the related compounds. This might result from the imprinting effect (Xu *et al.,* 2011) and acidity (Han *et al.,* 2010a). The result support the previous studied by Li et al., 2008 in which 2−HA−MISGs showed higher binding selectivity compared to 4−HA and sulfosalicylic acid. 2−HA exhibited higher acidity (pKa 2.98) compared to 3−HA (pKa = 4.08), 4−HA (pKa = 4.54) and phenol (pKa = 9.95) due to the ease of deprotonation (Huang *et al.,* 2003). This can be explained due to the fact that the structure of deprotonated 2−HA is very stable due to the formation double ring structure by intramolecular hydrogen bonding which can stabilize the conjugate base anion by resonance effect (Huang *et al.,* 2003). Hence, the interaction of 2−HA with the $–NH_2$ group is stronger than that of 3−HA, 4−HA and phenol.

Table 4.2: Competitive loading of 2−HA, 4−HA, 3−HA and phenol by the imprinted and non-imprinted sorbents

Sorbents		Imprinted	Non-imprinted
K_d	2-HA	4.257	0.485
	4-HA	0.3193	0.1207
	3-HA	0.2918	0.277
	phenol	1.05	0.2013
k	4-HA	13.33	4.018
	3-HA	14.59	1.751
	phenol	4.054	2.409
k'	4-HA	3.318	-
	3-HA	8.333	-
	phenol	1.683	-

distribution coefficient; $K_d = \{(C_i-C_f)/C_f\}x$ { volume of solution (mL)} / {mass of sorbent (g)}, where C_i and C_f represent the initial and final concentration respectively
selectivity coefficient, k = K_d (2−HA) / K_d (4−HA or 3−HA or phenol)
relative selectivity coefficient, k' = $k_{imprinted}/k_{non\text{-}imprinted}$
Initial concentration, C_i = 10 ppm
*results based on three replicate analyses for all analytes

4.3.5 Reusability

The sorbent should be reusable in order to make the sorbent economically competitive. The imprinted sorbent was used to extract 2−HA through five extraction cycles. The mixture solution of ethanol and HCl was used to strip the adsorbed 2−HA, then the material was filtered and neutralized with 0.1 M NaOH, and washed with deionized water. The results are shown in Table 4. Obviously, only very slight decrease of adsorption capacity occurred in reusability studies of the prepared 2−HA−MISG sorbent. This result indicated that the sorbent can be used repeatedly and the recognition sites were stable and as suggested by Yin *et al.*, (2012).

Table 4.3: Extraction recyclability through five extraction/stripping cycle

Extraction cycle	Loading capacity (mg/g)	Uptake (%)
1	4.123	82.88
2	4.317	86.34
3	4.335	87.13
4	4.298	86.39
5	4.354	87.07

4.3.6 Application to real samples

To validate the developed method, the recoveries of 2−HA at different spiking levels for the cosmetics samples (different brands of facial wash namely facial wash 1, 2, 3 and 4 respectively) and the results are listed in Table 4.4. The results showed that the recoveries of 2−HA were in the range from 86.89 % - 105 %. Relative standard deviation (RSD) values ranged from 0.05% to 0.97% in all cases. All the results indicated that the developed method has satisfactory accuracy and practical for the determination of 2−HA in cosmetic samples.

Table 4.4: Recovery of 2−HA in cosmetic samples at different spiking levels

Samples	spike (ppm)	Recovery (%) n=3	RSD (%) n=3
1	0.2	86.89	0.36
	1	93.02	0.61
2	0.2	98.27	0.05
	1	101.90	0.18
3	0.2	105.00	0.23
	1	96.20	0.97
4	0.2	91.20	1.60
	1	96.92	1.66

4.4 Effect of the temperature and contact time of 2-HA adsorption onto 2-HA-MISG

The equilibrium isotherms of 2-HA towards MISG sorbent at 298K, 323K and 353K are plotted in Figure 4.11. It was noted that the adsorption of 2-HA was rapid initially and then slowed down gradually until attaining the equilibrium. This suggested that possible monolayer coverage of 2-HA on the surface of the 2-HA-MISG. It can be seen that the adsorbed amounts decrease when temperature increases. This indicated that the adsorption of 2-HA onto sorbent was exothermic in nature. The result is supported by previous studies by Huang *et al.*, (2012) and Wang *et al.*, (2012).

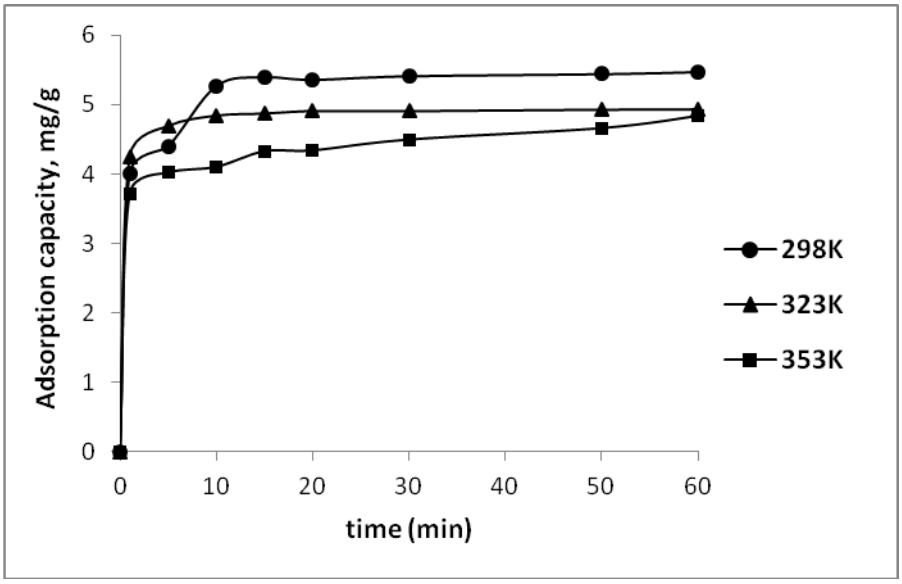


Figure 4.11: Effect of contact time on 2-HA adsorption onto 2-HA-MISG at different temperature

4.4.1 Adsorption kinetics

Different kinetic models were used to describe the rate of 2-HA uptake on the adsorbent in order to investigate the adsorption of the adsorbate on MISG adsorbent. The mechanism of adsorption and the potential rate-controlling steps involved mass transport and chemical reaction process (Ozacar and Sengil, 2005). The adsorption kinetics study was carried out to determine the time required for the adsorption equilibrium to be reached and condition under which there is no variation in adsorption capacity (Vidal *et al.,* 2011). In this study, the pseudo-first order and the pseudo-second-order kinetic were applied to the kinetics adsorption data to find the best fitted model for the experimental data.

4.4.2 Pseudo-first-order kinetic model

In many cases, the pseudo-first order kinetic model do not fit well to the whole range of the contact time, and is generally applicable at the initial stage of adsorption process (Wan *et al.,* 2010). The pseudo-first order model considers the rate of occupation of the adsorption sites proportional to the number of unoccupied sites (Chou *et al.,* 2011).

The pseudo first-order equation is expressed as:

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303} \qquad (8)$$

where q_e and q_t are amounts of 2-HA adsorbed (mg/g) at equilibrium and time,t (min), respectively, and k_1 is the rate constant of pseudo first-order (min^{-1}). The plots of log (q_e – q_t) versus t for the pseudo-first-order model given by Eq. (8) at temperatures of 298K, 323K and 353K were shown in Figure 4.12.

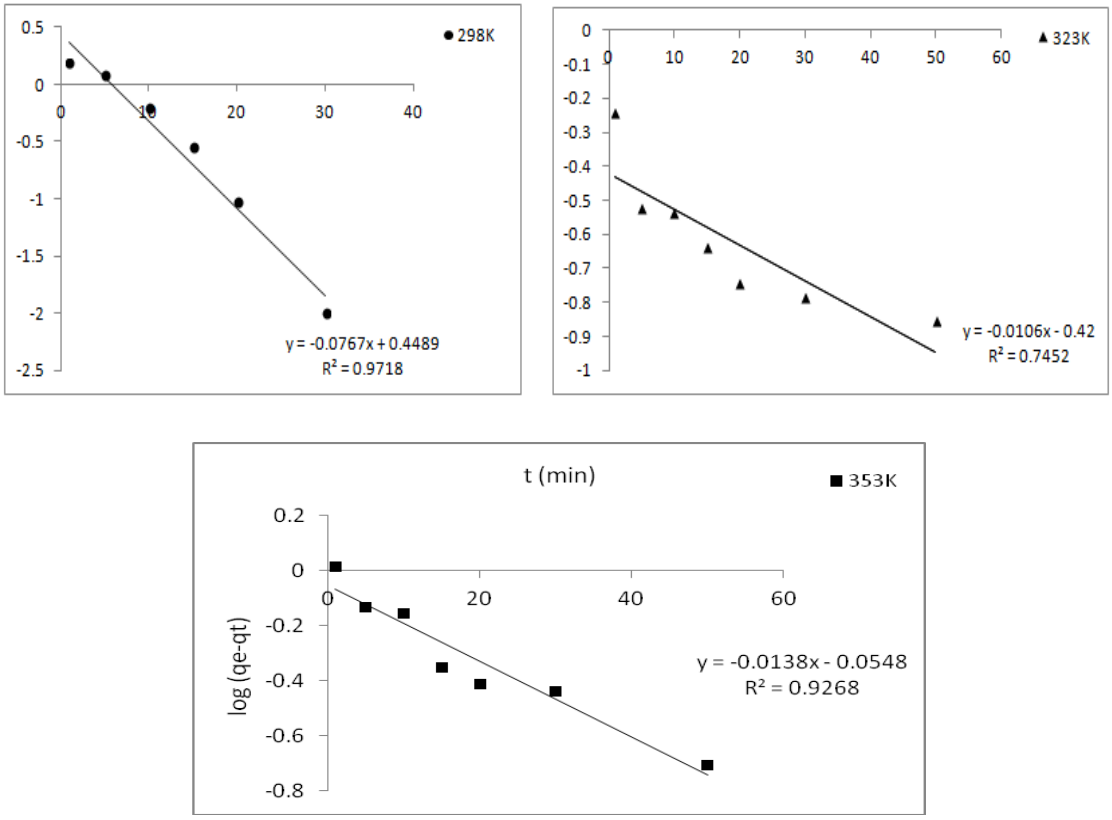


Figure 4.12: The pseudo first-order adsorption kinetics of 2-HA at different temperature

The k_1 and q_e were calculated from the slope and intercept of this plot. The k_1 values, q_e calculated and correlation coefficient are given in Table 4.5. The correlation coefficient (R^2) is low, ranging from 0.807 to 0.9836. The experimental, q_e values did not agrees with the calculated ones. This indicated that the adsorption of 2-HA did not follow the first order model.

4.4.3 Pseudo-second-order kinetic model

The pseudo second-order kinetic model which has been applied for analyzing chemisorptions kinetics from liquid solutions can be represented by Eq. (9) as follow:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^{\ 2}} + \frac{t}{q_e} \qquad (9)$$

where k_2 is the rate constant of pseudo second-order adsorption (g/mg min) and $h = k_2 q_e^{\ 2}$ where h is the initial adsorption rate (g/mg min).

The plots of (t/qt) versus t at different temperature were shown in Figure 4.13. The k_2 and q_e were calculated from slope and intercept of these plots,respectively.

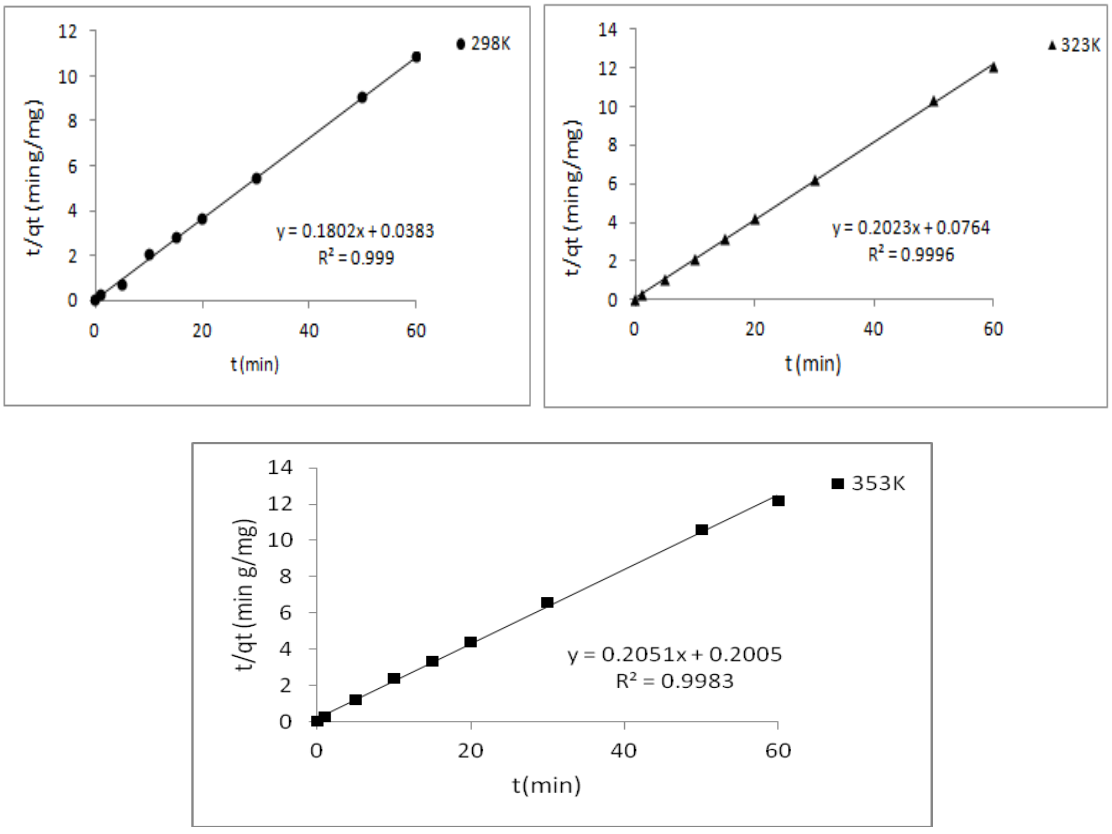


Figure 4.13: The pseudo second-order adsorption kinetics of 2-HA at different temperature

As can be seen from Table 4.6, the correlation coefficients (R^2), for the second-order kinetic model are between 0.9983 and 0.999 and the experimental q_e, exp values

agree with the calculated ones. This indicated that the adsorption process fits well to the pseudo-second order model and involves chemisorption process (Abramian and El-Rassy, 2009). This suggested that chemisorption might be the rate limiting step controlling the 2-HA-binding process which is the process involved two stages comprising of a fast initial phase and a second slower phase. In the first phase, the 2-HA molecules are adsorbed on the external sites of the sorbent material in a fast process which dominates the initial kinetics of adsorption. In the second phase, the molecules of 2-HA slowly diffuse into the pores of the material and are adsorbed onto the internal sites as suggested by Hamdaoui and Chica, (2007) for the removal of methylene blue by wheat bran.

Table 4.5: Kinetic parameters for 2-HA adsorption onto 2-HA-MISG

Temperature (K)	298	323	353
q_e, exp (mg/g)	5.541	4.985	4.917
Pseudo first-order equation			
q_c (mg/g)	2.811	0.3802	0.8815
k_1 (min^{-1})	0.1766	0.02441	0.003178
R^2	0.9718	0.7452	0.9268
Pseudo second-order equation			
q_c (mg/g)	5.549	4.943	4.876
k_2 (g/ mg min)	0.8479	0.5357	0.2098
R^2	0.999	0.9996	0.9983

4.5 Effect of the temperature and concentration of 2-HA adsorption onto 2-HA-MISG

The effect of temperature on 2-HA adsorption was investigated by measuring the adsorption isotherms in the temperature range 298K to 353K using initial 2-HA concentration of 20-300 mgL^{-1} as shown by Figure 3b. The equilibrium uptake of 2-HA increased with increase in the initial 2-HA concentrations for all temperatures. For example, the adsorption capacity of 2-HA increased from 8.57 – 76.2 mg/g, 4.67 –58 mg/g, 3.25 – 45.35 mg/g at 298K, 323K and 353K respectively. This occurs due to higher probability of collision between 2-HA molecule and 2-HA-MISG surface (Noelin *et al.*, 2005).

It can be observed from the Figure 3a and 3b that the equilibrium uptake of 2-HA molecules by 2-HA-MISG decreased with increase in temperature from 293K to 323K indicating that physical adsorption takes place. This suggests that the process is exothermic and the lower temperature (298K) is more favorable for the adsorption 2-HA. In physical adsorption, the equilibrium is usually rapidly attained and easily reversible because the requirements of energy are small (Aksu, 2002). The adsorption was low at higher temperatures between 298K and 353K due to the decrease in physical force for adsorption or due to the decrease in the intraparticle diffusion rate of 2-HA into the 2-HA-MISG (Jadhav and Vanjara, 2004).

Furthermore, the decrease in the percentage of 2-HA removal at higher temperature may be attributed to the deactivation of the sorbent surface or the destruction of some polymeric active sites on the adsorbent surface due to the bond rupture (Ozsoy *et al.*, 2008). Consequently, the study found that the 2-HA adsorption process was affected by temperature and that the optimum working range was 298K. These results are in agreement with those described by Wang *et al.,* (2012) and Huang *et al.,* (2012).

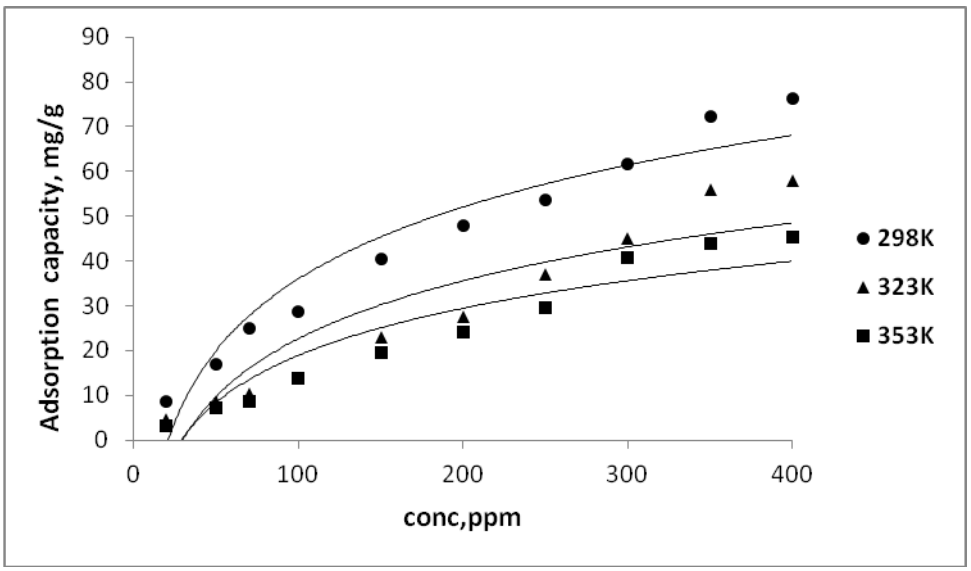


Figure 4.14: Effect of concentration on 2-HA adsorption onto 2-HA-MISG at different temperature

4.5.1 Adsorption isotherm

Adsorption isotherms describe how adsorbate interacts with the adsorbents (Wang *et al.,* 2010) and therefore, it is a priority to optimize the design of an adsorption process. Hence, it is essential to establish the most appropriate correlation for the equilibrium curve. Several models have been used in the adsorption studies to describe the experimental data on adsorption isotherms. Langmuir and Freundlich isotherms are the most frequently used models and were investigated in this work. Different equation parameters and the underlying thermodynamics presuppositions of these models often provide insight into both adsorption mechanism and surface properties of the adsorbent (Renault *et al.,* 2008).

4.5.2 Langmuir isotherm

The Langmuir model was originally developed to represent chemisorption at a set of well-defined localized adsorption sites with same adsorption energy, independent of the surface coverage and no interaction between adsorbed molecules. This model assumes a monolayer deposition on a surface with a finite number of identical sites. It is well known that the Langmuir equation is valid for a homogenous surface (Chou *et al.*, 2011). Therefore, at equilibrium, a saturation point is reached where no further adsorption can occur (Elkady *et al.*, 2011).

The linearized Langmuir isotherm is represented by following equation:

$$\frac{C_e}{q_e} = \frac{1}{q_m b} + \frac{C_e}{q_m} \qquad (10)$$

where C_e is the 2-HA concentration at equilibrium (mg/L), qe is the adsorption capacity at equilibrium (mg/g), b is the Langmuir binding coefficient (L/mg) and q_m is the maximum binding capacity (mg/g). From a plot of C_e/q_e versus C_e, b and q_m can be determined from the slope and intercept of the resulting straight line.

Figure 4.15 shows the plot of 2-HA adsorption isotherm in Langmuir model sorption at different temperature (298K, 323K, 353K) with the correlation coefficient, R^2 is 0.5537, 0.4201, and 0.5026 respectively. The values of q_m and b for Langmuir isotherm are listed in Table 4.6. It was found that the plot is not linear with good correlation coefficient; hence the adsorption process is not suitable for Langmuir isotherm.

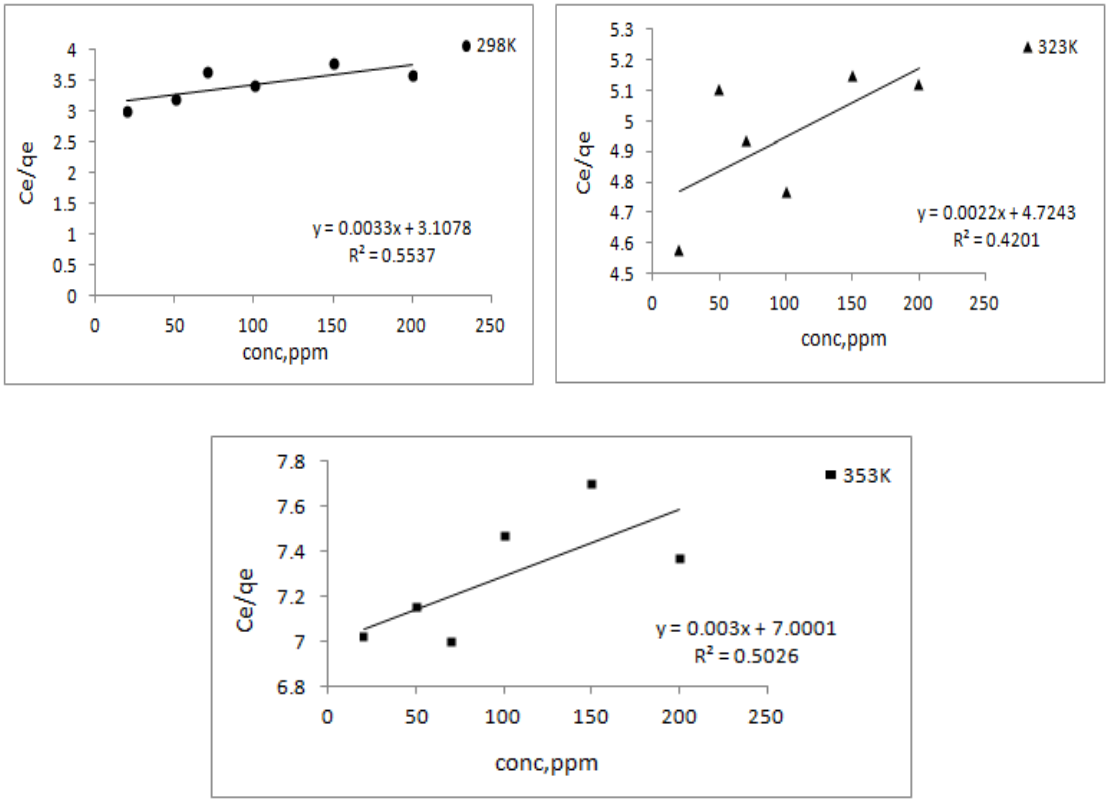


Figure 4.15: Langmuir adsorption isotherm of 2-HA-MISG at different temperature

4.5.3 Freundlich isotherm

The Freundlich model applicable to adsorption on heterogenous surfaces with the interaction between adsorbed molecules and the application of the equation suggests that the adsorption energy exponentially decreases on completion of the adsorption centres of adsorbent (Greluk and Hubicki, 2010).

The isotherm is adopted to describe the reversible adsorption and is not restricted to monolayer formation (Chou *et al.*, 2011). The Freundlich adsorption isotherm can be expressed as:

$$\log q_e = \log K_f + \frac{1}{n}\log C_e \qquad (11)$$

where the intercept, log K_f is a measure of adsorbent capacity, the better sorption corresponds to higher K_f values and the slope 1/n is the adsorption intensity, n is related to the magnitude of the sorption driving force and to the sorbent site energy distribution. Figure 4.16 shows the 2-HA adsorption isotherm followed the linearized Freundlich model at different temperature (298K, 323K, and 353K).

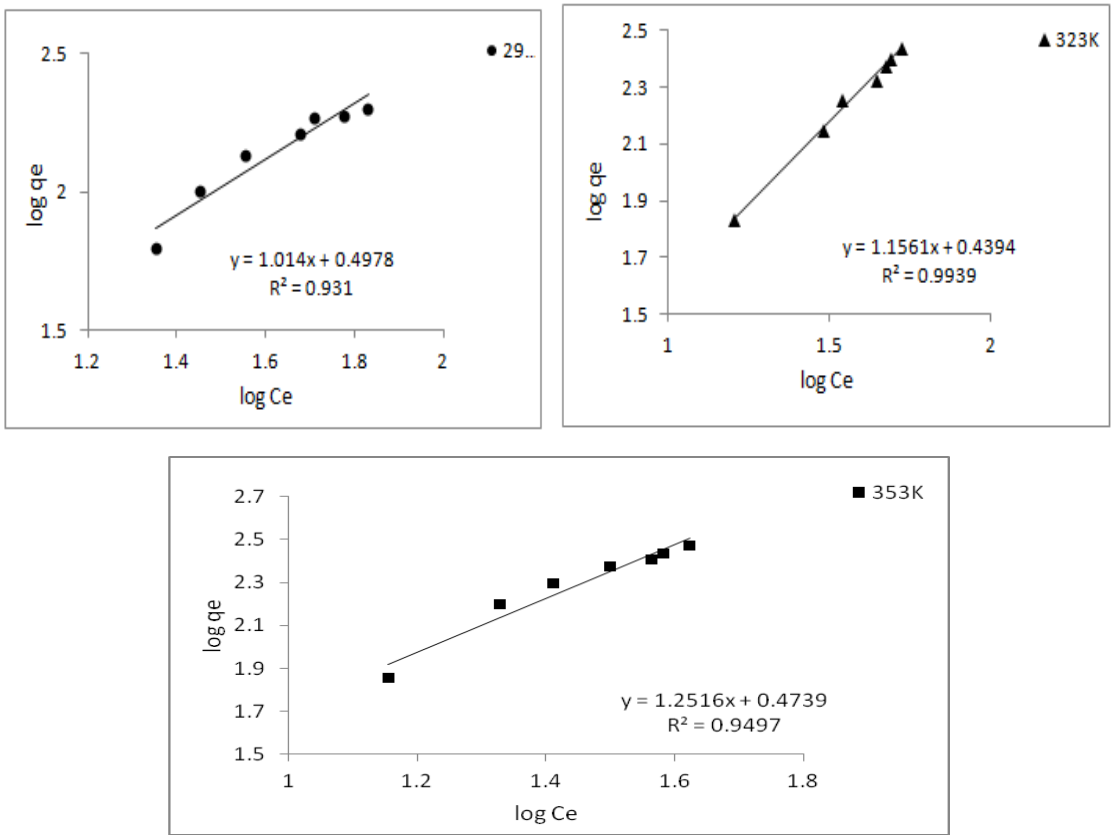


Figure 4.16: Freundlich adsorption isotherms of 2-HA-MISG at different temperature

Values of K_f and n can be calculated by plotting log (q_e) versus log (C_e). The slope of the linear plot is equal to n and the intercept is equal to log (K_f). From Fig. 4.16, a linear relation was observed among the plotted parameters at different temperatures. A comparison of the isotherm constants along with regression coefficient (R^2) is presented in Table 4.6. The values of correlation coefficient (R^2) are found to be > 0.9 for this isotherm.

According to the R^2 values, it is found that the Freundlich model fitted the experimental data better than the Langmuir model for the description of the adsorption isotherm of 2-HA onto silica gel sorbent. Obviously, there was a decrease in the K_f values as the temperature increased for 2-HA which indicated that sorption of 2-HA towards sorbent was better at lower temperatures. This was in good agreement with observations from Figure 4.14.

The Freundlich isotherm has been illustrated to be a special case of heterogeneous surface energies however it can be easily extended to this case. In most cases the exponent between $1 < n < 10$ shows beneficial adsorption (Annadurai *et al.*, 2008; Ho & Mc Kay, 2000). When the value of n is bigger than 1, chemical adsorption process occurs naturally. The physical adsorption takes place when the values of n is lower than 1 (Wu, 2007). The case where $n < 1$ reflects the situation in which at higher adsorbate concentrations, it becomes more difficult to adsorb additional molecules. This could occur in cases where specific binding sites become filled or remaining sites are less attractive to the adsorbate molecules. The case where $n > 1$ describes a contrast situation, in which previously adsorbed molecule lead to a modification of the surface which favors further adsorption (Schwarzenbach *et al.*, 1993).

The value of n for 2-HA adsorption on 2-HA-MISG for different temperatures 298K, 323K and 353K were as follows 0.9862, 0.8649 and 0.7989 respectively (see table 4.6). These values indicated that the conditions of adsorption process were favourable and physical adsorption took place for the removal of 2-HA.

Table 4.6: Isotherm parameter for 2-HA adsorption onto 2-HA-MISG

Isotherm models	298K	323K	353K
Langmuir			
q_m (mg/g)	64.35	42.33	28.57
b (L/mg)	4.709	10.74	11.67
R^2	0.5537	0.4201	0.5026
Freundlich			
K_f (mg/g)	3.146	2.978	2.7504
n	0.9862	0.8649	0.7989
R^2	0.931	0.9939	0.9497

4.6 Adsorption thermodynamics

It is assumed that in an isolated system, energy cannot be gained or lost and the entropy change is the only driving force based on fundamental thermodynamics concept (Kumar and Kumaran, 2005). The effect of temperature on the adsorption of 2-HA onto silica gel sorbent can be determined by calculating the free energy change (ΔG°), enthalpy change (ΔH°), and entropy change (ΔS°). The adsorption process can be summarized to represent a heterogeneous equilibrium. The Gibbs free energy indicates the degree of spontaneity of the adsorption process and the higher negative value reflects a more energetically favorable adsorption (Aksu, 2002). The change in Gibbs free energy, ΔG° of the adsorption was calculated according to the following Eq. 12. :

$$\Delta G° = - RT \ln K_c \quad (12)$$

where R is gas constant, 8.314 J mol^{-1} K^{-1}, K_c is the equilibrium constant and T is temperature in K.

The K_c values are calculated from Eq. 5:

$$K_c = \frac{C_{ad,eq}}{C_{eq}} \tag{13}$$

where $C_{ad,eq}$ and C_{eq} is the equilibrium concentration of 2-HA on adsorbent (mg/g) and in the solution (mg/L), respectively.

In the other hand, ΔG° is determined from the following equation:

$$\Delta G° = \Delta H° - T\,\Delta S° \tag{14}$$

The combination of Eq (12) and (14) leads to the van't Hoff equation as follows:

$$\ln K_c = -\frac{\Delta H°}{RT} + \frac{\Delta S°}{R}$$

The slope and intercept of the van't Hoff plot is equal to - $\Delta H°/R$ and $\Delta S°/R$ respectively. The van't Hoff plot for the adsorption of 2-HA onto 2-HA-MISG is given in Figure 4.17.

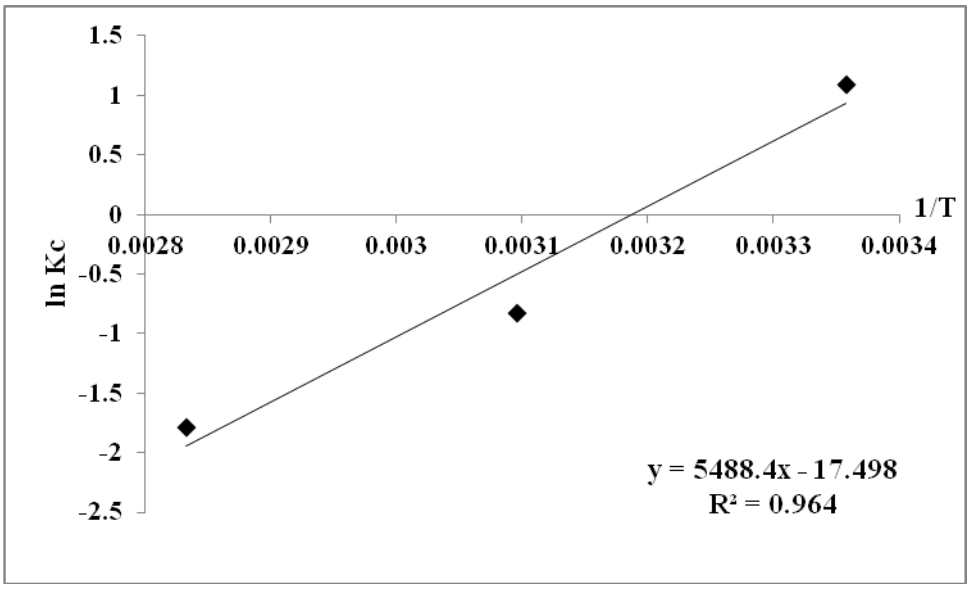


Figure 4.17: Van't Hoff plots of 2-HA adsorption onto 2-HA-MISG

The calculated thermodynamic parameters are presented in Table 4.7. The standard Gibbs free energy, ΔG° for the adsorption process was obtained as – 2.708, 4.804, and 2.428 kJ/mol at 298K, 323K and 353K, respectively. The negative values of ΔG° at different temperature suggested that adsorption process leads to a decrease in Gibbs free energy. This confirms the feasibility and spontaneous nature of adsorption (Rodriquez *et al.,* 2009). The negative values of ΔG° indicated that adsorption process is exothermic and spontaneous nature at all temperatures. Higher negative values of ΔG° reflect more energetically favorable sorption. The positive values of ΔG° at 323K and 353K indicate the existence of energy barrier for the 2-HA adsorption. It also suggests that an input of energy is required for reactant molecules to have sufficient kinetic energy to overcome the energy barrier (Mahmood *et al.,* 2011). Therefore, adsorption of 2-HA on sorbent at 298K was decided the most favorable adsorption process. Furthermore, the absolute magnitude of the change in free energy for adsorption is between 0 and -20 kJ/mol. This suggests that adsorption type can be explained as physisorption (Gereli *et al.,* 2006).

The negative values of ΔH° suggested that the 2-HA adsorption is an exothermic process. There are no certain criteria about the ΔH° values that define the adsorption type. According to Karaca *et al.,* 2004 the ΔH° is lower than the value of 40 kJ/mol suggested that the process is physical adsorption.

The negative values of ΔS° suggested the decrease of randomness at the solid-solution interface during the 2-HA adsorption of aqueous solutions onto adsorbents. As the temperature increases, the mobility of 2-HA molecule increases causing the molecule to escape from the solid phase to the liquid phase. Therefore, the amount of 2-HA that can be adsorbed will decrease (Wan Ngah and Hanafiah, 2008). However, it should be also noted that the entropy of the universe (including the system and the surroundings) might be increase due to the adsorption reaction was not an isolated process (Li *et al.*, 2012).

Table 4.7: Thermodynamic parameters for the adsorption of 2-HA onto 2-HA-MISG

Temperature (K)	ΔG° (kJ/mol)	Δ H° (kJ/mol)	ΔS° (J/molK)
298	-2.708		
323	4.804	- 45.63	- 145.5
353	2.428		

5 Conclusions

The removal of 2-HA from aqueous solution was studied by using molecularly imprinted silica gel (MISG) sorbent. In this study, a simple molecular imprinting procedure was adopted to synthesize a highly selective 2-HA-imprinted silica gel sorbent by combining a surface molecular imprinting technique with a sol gel process. The prepared imprinted material showed good characteristics such as fast adsorption, high affinity, selectively and can be regenerated for 2-HA. The result showed the potential of molecularly imprinted silica sorbent for selective removal of 2-HA in real samples. The adsorbed amount of 2-HA increase with increasing contact time until reaching the equilibrium at 60 minutes. It shows that the adsorption process favours at lower temperature (298K) which indicates that the process is exothermic in nature. The adsorption of 2-HA increase with the increasing initial concentration of 2-HA solution at lower temperature (298K). The kinetics of adsorption of 2-HA on the sorbent from aqueous solution fitted well with the pseudo second-order model indicating the chemisorptions as the rate limiting step. The equilibrium isotherm data are best fitted with the Freundlich isotherm confirming that the multilayer adsorption of 2-HA on sorbent. The negative value of $\Delta G°$ confirms the spontaneous nature of the adsorption process. Only the positive value of $\Delta G°$ obtained at a temperature of 323K and 353K, indicating the presence of an energy barrier is the exception to this rule. The negative value of ΔH° indicates that the 2-HA adsorption is an exothermic process. The negative value of ΔS° shows that the randomness at the solid-solution interface is decrease during adsorption.

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